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1-Alpha-hydroxycholecalciferol Treatment of Adults with Chronic Renal Failure

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ABSTRACT Five adult patients with chronic renal failure and associated renal osteodystrophy have been treated for 6 months with 1 alpha hydroxy cholecalciferol (1α OH D_3), a synthetic vitamin D analogue All 5 patients had severe metabolic bone changes as estimated by bone scintigraphy Three patients were hypocalcemic, 4 had elevated serum alkaline phosphatases 5 had elevated serum immuno reactive parathyroid hormone (i PTH) concentration and 3 had bone pains During treatment serum calcium increased in all patients (mean 11 4%) and 3 originally hypocalcemic patients became normocalcemic Serum alkaline phosphatases decreased (mean 27 3%) and became normal in 4 patients, who initially had elevated values A pronounced decline in the serum concentration of i PTH (mean 53%) was seen in all patients and 1 patient obtained normal i PTH levels after 4 months of treatment The intestinal calcium absorption which was low initially, even when calcium intake was considered, rose almost threefold (mean 273%) and reached normal values in all cases The bone mineral content increased in all patients, but the changes were small (mean 4 9%) and insignificant Finally, bone pain disappeared in 2 patients and improved in 1 of the 3 patients exhibiting this symptom A linear correlation (r=0 48, p<0 001) was found between the dose of 1α OH D_3 and serum calcium But in spite of this and the frequent control, all patients developed one episode of hypercalcemia This disappeared within 48 hours after discontinuing the drug It is concluded that treatment with 1α OH D_3 appears to be of therapeutic value in metabolic bone disease associated with chronic renal failure, but frequent control of blood biochemistry seems mandatory

Vitamin D is converted by enzymatic hydroxylations on carbon atom 25 in the liver (17) and subsequently on carbon atom 1 in the kidneys (9) to form 1α 25-dihydroxy vitamin D_3 (1α 25 (OH) D_3) This metabolite is the most potent vitamin D known (10 16 19) and should be regarded as a hormone (13) being produced exclusively in the kidneys and transported via the circulation to exhibit its major biological functions in the intestine (14) and in bone (20)

1α 25-$(OH)_2$ D_3 can be synthesized (18) but the synthesis has proved difficult and expensive making the need for a more convenient analogue obvious Using cholesterol as starting material 1α hydroxy vitamin D_3 (1α OH D_3) was synthesized in 1973 (12) The production of this analogue is relatively simple and the biological potency has proven to be about one half of that of 1α 25 $(OH)_2$ D_3 (11) The mechanism of action of 1α OH D_3 is not definitely known but it has been suggested (21) that the analogue is converted in the liver to the genuine hormone 1α 25 $(OH)_2$ D_3 As 1α OH D_3 may by pass the hydroxylation on carbon atom 1 which is impaired in patients with chronic renal disease (15) it seems theoretically rational to treat patients with renal osteodystrophy with this compound

Two groups of investigators (4 6) each treating 3 adults with renal osteodystrophy with 1α OH D_3 recently reported encouraging results The period of treatment did not exceed 3 months in these reports Based on the results of the treatment of 5 adults for 6 months this paper confirms the beneficial effect of 1α OH D_3 in renal osteodystrophy

MATERIAL

Five patients (2 females and 3 males) with a mean age of 39 years (range 24–56) were studied (Table I) The approximate duration of severe renal failure (creatinine clearance below 20 ml/min) was 6 years (range 1 5–10) One patient

Table I *Clinical data of 5 uremic adults receiving 1α-OH D₃*

Pat no	Age (y)	Sex	Duration of uremia (y)	Renal disease
1	24	♂	1½	Chronic glomerulonephritis
2	38	♀	4	Chronic interstitial nephropathy
3	56	♀	10	Chronic interstitial nephropathy
4	30	♂	10	Congenital nephropathy
5	45	♂	4	Malignant nephrosclerosis

(no 5) received regular hemodialysis while 4 patients had severely impaired renal function with creatinine clearances not exceeding 6 ml/min The renal diseases are listed in Table I

The cases were selected among uremic patients who fulfilled 4 of the following 5 criteria 1) Hypocalcemia i e sustained serum calcium below 2 30 mmol/l ($n=3$) 2) Elevated serum alkaline phosphatases i e values permanently exceeding 275 U/l (normal range 50–275) ($n=4$) 3) Elevated serum concentration of immunoreactive parathyroid hormone (i PTH) i e values exceeding 2 5 ng/ml (normal range 1 1–2 5) ($n=5$) 4) Severe grade III (22) symmetrical bone changes evaluated by ^{99m}technetiumpolyphosphate whole body scintigraphy (^{99m}Tc PP) ($n=5$) 5) Clinical symptoms i e pain definitely attributable to the bones ($n=3$) The individual patient fulfilled the cri[illegible] shown in Table II

METHODS

The patients received 1α OH D₃ (supplied by Leo Pharmaceuticals Copenhagen) dissolved in propylene glycol orally for approximately 6 months (mean 180 days) The dose of 1α-OH D₃ was 0–2 μg/day and was reduced if hypercalcemia (serum calcium exceeding 2 7 mmol/l) developed or if the Ca×P product exceeded 6 (mmol/l)² During the study serum phosphate was kept within or close to the normal range (0 80–1 48 mmol/l) with an oral phosphate binder (aluminium aminoacetate)

The 5 patients served as their own controls All i[illegible] vestigations were carried out before as well as duri[illegible] treatment During the month preceding the start of [illegible] ment and after approximately 6 months of trea[illegible] (mean 180 days) the following investigations were c[illegible] out total serum calcium serum ionized calcium [illegible] phosphate serum magnesium serum alkaline [illegible] phatases serum i PTH osteodensitometry Tc PP [illegible] graphy X ray examination of the skeleton and me[illegible] ment of the intestinal calcium absorption

The analysis of PTH in serum was performed b[illegible] Stockholm Immunlaboratory AB (1) and osteode[illegible] metry by photon absorptiometry according to Ca[illegible] and Sorenson (2) using ^{241}Am as photon source The [illegible] mineral content (BMC) of the right forearm wa[illegible] pressed in g/cm The Tc PP scintigraphy was perf[illegible] as previously described (22) while calcium abso[illegible] was measured as described by Curtis et al (8) using [illegible] volume scintillation counter (Armac Packard Instr[illegible] Co) The absorption was calculated as the perce[illegible] of the orally administered ^{47}Ca present in the forearm [illegible] 23 hours expressed in relation to the amount o[illegible] present in the forearm 23 hours after i v administr[illegible] In our hands the standard deviation for duplicate e[illegible] tions in 11 normal persons was 2 6%

During the period of treatment the patients were [illegible] uated clinically once a week and the calcium and [illegible] phate concentrations in serum were measured [illegible] taneously

RESULTS

Fig 1 demonstrates the principal results o[illegible] treatment in patient 1 suffering from chroni[illegible] merulonephritis and with a creatinine clearan[illegible] 5 ml/min It appears that this patient initially h[illegible] calcemic became normocalcemic on 1α OH [illegible] μg/day and developed hypercalcemia over 8 w[illegible] when the dose was increased to 2 μg/day [illegible] hypercalcemia disappeared rapidly when adm[illegible] tration of the drug was discontinued The pa[illegible] showed initially a moderate degree of phos[illegible] retention which exaggerated when 1α OH D[illegible] given When the dose of aluminium aminoac[illegible] was increased (indicated by an arrow in Fig 1[illegible] hyperphosphatemia could be controlled but a [illegible]

Table II *Criteria of selection before treatment with 1α-OH D₃*

Pat no	s Ca↓	Alk phosph ↑	i PTH↑	Tc scintigraphy group III	Clinical symptoms
1	+	+	+	+	
2	+	+	+	+	
3		+	+	+	+
4	+		+	+	+
5		+	+	+	+

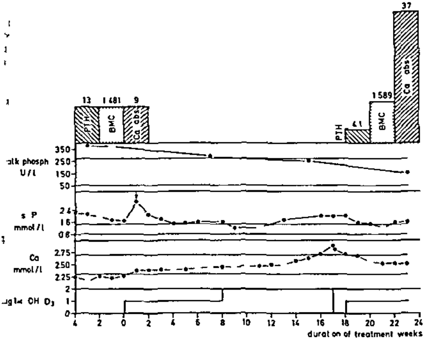


Fig 1 Results from 160 days treatment with 1α OH D_3 in a 24 year-old male with *chronic nephropathy (patient I)* *PTH*=immunoreactive parathyroid hormone concentration (ng/ml) *BMC*=bone mineral content (g/cm) Ca abs=intestinal calcium absorption (%)

tendency to hyperphosphatemia was seen at the time of the hypercalcemia The alkaline phosphatases being moderately increased at the start declined gradually and became normal during the treatment The grossly elevated i PTH concentration (13 ng/ml) declined while a small and insignificant increase in BMC was seen The calcium absorption initially pathologically reduced even when the calcium intake was considered increased to normal levels

In the total material (Fig 2) the concentration of alkaline phosphatases decreased by 27 3% and became normal The BMC increased in all patients (mean 4 9%) although not significantly The i PTH concentration declined by 53% and the intestinal calcium absorption rose almost threefold (273%) The X ray survey of the skeleton did not reveal focal lesions but in 3 patients osteoporosis was present This was in accordance with the results of the osteodensitometry Roentgenographically the patients remained unchanged Finally although objective measurements are not available it appeared convincing that bone pain disappeared in 2 patients and improved in 1

During the period of treatment serum calcium was measured once a week A linear correlation was found between the dose of 1α OH D_3 and the serum calcium response ($n=125$ $r=0\ 48$ $p<0\ 001$) (Fig 3) Serum calcium increased in every patient (mean 11 4%) and this was most pronounced in the 3 hypocalcemic patients who became normocalcemic during the treatment Serum ionized calcium increased in a parallel manner while serum magnesium remained unchanged The percentage changes in serum calcium serum alkaline phosphatasis i PTH concentration bone mineral content and intestinal calcium absorption in each patient are shown in Table III

In spite of frequent control all patients developed one episode of hypercalcemia In no case was this followed by clinical symptoms and the hypercalcemia disappeared in every case within 48 hours after discontinuing the administration of the drug Apart from this hypercalcemia no side-effects were observed

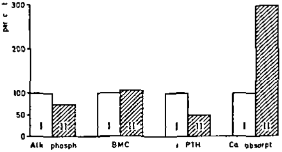


Fig 2 Percentage changes in 5 patients before (*I*) and after (*II*) 6 months treatment with 1α OH D_3

Table III *Percentage changes in each patient after treatment with 1α-OH D₃*

Pat no	s Ca	s alk phosph	S i PTH	BMC	Calcium absorption
1	+16 7	−53 2	−76 9	+ 7 3	+293 8
2	+13 5	−17 7	−33 7	+ 1 0	+169 6
3	+ 8 6	−32 1	−50 6	+ 2 2	+428 5
4	+16 0	− 6 3	−46 8	+ 0 3	+ 52 4
5	+ 3 2	− 6 2	−36 4	+11 1	+ 48 2

DISCUSSION

In the present investigation on 5 adult patients important bone related parameters showed a tendency to normalize during treatment with 1α OH D_3

The linear correlation demonstrated between the dose of 1α OH D_3 and serum calcium suggests that the treatment is reasonably controllable In spite of that all 5 patients developed hypercalcemia once during the treatment indicating that careful and continuous control of serum calcium and prevention of phosphate retention are imperative during this treatment The rapid disappearance of hypercalcemia after discontinuing the drug makes the treatment superior to conventional vitamin D treatment also in this respect

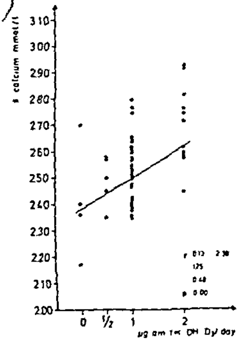


Fig 3 Correlation between the dose of 1α OH D₃ and the serum calcium concentration

The suppression of i PTH found here may [cut] direct effect of 1α OH D_3 (or 1α 25-(OH)$_2$ D_3) [cut] indirect effect through the increase in serum [cal]cium or some combination of these This [im]portant problem is unsolved and deserves fu[rther] attention

A therapeutic effect of 1α OH D_3 in adolesc[ents] with renal osteodystrophy has recently been [re]ported (7) The results of the few reports (4 [cut] concerning 1α OH D_3-treatment of adults [cut] metabolic bone disease associated with chr[onic] renal failure are in accordance with the data of [the] present investigation in most aspects The incr[ease] in BMC in our patients did not reach statist[ical] significance while Catto et al (4) using neu[tron] activation technique (5) found a significant incr[ease] after 3 months The latter technique may be a m[ore] sensitive indicator of the rate of improvemen[t] renal bone disease than photon absorptiometry [cut] explaining this quantitative discrepancy

Based on the beneficial effect of 1α OH D_3 [cut] important bone related parameters it is conclu[ded] that this compound may be of therapeutic valu[e] diseases in which a failure of 1 hydroxylatio[n] vitamin D is present Long term studies on la[rger] patient materials are needed to determine the [de]finite place of 1α OH D_3 in the therapy of r[enal] osteodystrophy

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Renal Handling of Phosphate in Relation to Serum Parathyroid Hormone Levels

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ABSTRACT The relation between the renal handling of phosphate expressed as the maximal tubular reabsorption of phosphate (TmP)/glomerular filtration rate (GFR) index and the serum concentration of immunoreactive parathyroid hormone (i PTH) has been investigated in 15 patients with a very wide range of GFR TmP/GFR and i PTH Seven patients had well functioning kidney allografts, with GFR ranging from 43 1 to 64 9 ml/min, while eight had varying degrees of chronic nephropathy, with GFR ranging from 26 7 to 2 3 ml/min The TmP, the i PTH concentration, the ^{51}Cr EDTA clearance the extracellular volume and the serum concentrations of calcium and standard bicarbonate were estimated during conditions where tubular reabsorption of phosphate was maximal An inverse significant correlation was demonstrated between TmP/GFR and i PTH ($p<0.001$), while none of the other investigated factors correlated to the TmP/GFR index It is therefore concluded that the parathyroid hormone has a key role in the regulation of the tubular handling of phosphate in patients with impaired renal function

The maximal tubular reabsorption of phosphate (TmP) is highly correlated to the glomerular filtration rate (GFR) (5) and therefore to reduce the contribution of GFR to the variation of TmP the latter must be expressed in relation to GFR This TmP/GFR index appears to be the most consistent index of renal phosphate handling (25)

It is well established that parathyroid hormone (PTH) causes phosphaturia in patients with normal renal function by depressing the renal tubular reabsorption of phosphate (10) As radioimmunoassay of PTH is now available we have found it of interest to investigate whether a correlation exists between TmP/GFR and the serum level of immunoreactive PTH (i PTH) in a group of patients with a wide range of renal function and serum levels of i PTH

MATERIAL

The material consists of 15 patients (8 females 7 males) with an age range of 19–58 years (mean 38) Seven patients (4 females 3 males) had well functioning kidney allografts with creatinine clearances of 43–65 ml/min (mean 59) Their age range was 22–58 years (mean 35 6) The average time of the present investigation after the kidney transplantation was 18 months (range 6–60) The kidney transplanted patients received a daily prednisone dosage of 0 (one patient)–25 mg (mean 12 9) None of these patients showed clinical or biochemical signs of rejection at the time of the investigation Eight patients (4 females 4 males) had varying degrees of chronic progressive renal insufficiency with creatinine clearances of 27–2 ml/min (mean 10 6) The age range was 19–58 years (mean 40 5) No patient received dialysis treatment The basic nephrological diseases were chronic glomerulonephritis in 2 chronic interstitial nephropathy in 2 polycystic kidney disease in 2 and congenital nephropathy in 2 patients The treatment with oral phosphate binder (aluminium aminoacetate) in these patients was withdrawn 48 hours prior to the study and in the entire group any diuretic treatment was withdrawn 24 hours before the study No patient received vitamin D treatment

METHODS

The investigations were carried out at 9 a m The seven kidney transplanted patients who were normo- or slightly hypophosphatemic (serum phosphate range 0 72–1 04 mmol/l) received an i v phosphate infusion at the start of the study in order to obtain maximal renal phosphate reabsorption The infusion consisted of a 0 1 M solution of phosphate buffered at pH 7 4 (Na_2HPO_4 14 4 g KH_2PO_4 2 58 g water to 1 l) delivered at a rate of 100/ml hour with an infusion pump (Braun Infusomat®) After 60 min the seven patients were hyperphosphatemic (serum phosphate range 1 74–2 47 mmol/l) and during continuous phosphate infusion a urine sample was collected in the following 120 min period (All patients could void on re

Table I *Measurements of glomerular filtration rate (GFR) maximal tubular reabsorption of phosph (TmP) TmP/GFR index serum concentration of immunoreactive parathyroid hormone (i PTH) serum phosphate and serum calcium concentration*

Pat no	GFR (ml/min)	TmP (μmol/min)	TmP/GFR (μmol/ml)	i PTH (ng/ml)	Se P (mmol/l)	Se-Ca (mmol/l)
Kidney transplanted patients						
1	43 1	36 9	0 85	2 1	1 74	2 68
2	64 9	50 2	0 77	1 9	1 89	2 55
3	57 3	42 5	0 74	1 9	2 01	2 48
4	62 4	45 3	0 72	2 4	2 11	2 67
5	61 8	42 9	0 69	2 8	1 92	2 41
6	60 1	34 9	0 58	3 3	2 15	2 39
7	62 1	24 0	0 38	4 3	2 47	2 60
Patients with chronic nephropathy						
8	8 1	5 7	0 70	1 9	2 56	2 27
9	26 7	18 3	0 69	1 4	2 47	2 38
10	6 2	1 9	0 46	4 1	2 19	1 95
11	18 1	7 7	0 42	3 0	2 05	2 17
12	11 7	4 1	0 35	3 5	1 62	2 08
13	4 1	1 1	0 11	9 5	1 81	2 33
14	2 3	0 2	0 08	4 7	1 67	2 29
15	7 5	0 5	0 03	13 0	1 97	2 49

quest) Blood samples were collected every 30 min during the urine sampling and analyzed for phosphate The average serum phosphate in each 120 min period was used in the calculations At the beginning of the period of urine sampling a blood sample was collected and analyzed for PTH and serum calcium

The eight patients with impaired renal function all had a nsiderable degree of phosphate retention (serum phosphate range 1 62–2 56 mmol/l) so the renal phosphate reabsorption could be regarded as maximal and no phosphate was administered during the investigation

GFR was estimated during the study using the single injection technique of [51]Cr EDTA (16) and similarly the extracellular volume was calculated as the [51]Cr EDTA distribution space (11) Phosphate in plasma and urine was measured as described by Dryer et al (6) all phosphate values being expressed as mmol/l and the TmP (μmol/min) in the 120 min periods was calculated as the difference between filtered (GFR×mean serum phosphate) and excreted phosphate (urine phosphate×urine volume/min) The analysis of PTH in serum was performed by the Stockholm Immunlaboratory AB using a radioimmunoassay based on the ability of *human PTH to compete with [125]I labelled bovine PTH for* binding to a guinea pig antiserum directed against bovine PTH (2) Normal subjects had a range from 1 1 to 2 5 ng bovine PTH equivalents/ml Finally serum standard bicarbonate was estimated in each patient before the start of the investigation

RESULTS

The values of GFR TmP TmP/GFR and i PTH as determined in the 15 patients are given in Table I The GFR values ranged from 2 3 to 64 9 ml/min (mean 33 1) in the group of eight patients with chronic nephropathy from 2 3 to 26 7 ml/min (mean 10 6) and in the group of seven kidney transplanted patients from 43 1 to 64 9 ml/min (mean 58 8) The TmP values (mean 44 8 μmol/min) were significantly higher (p<0 001) in the transplanted patients than in the patients with chronic nephropathy (mean 4 9 μmol/min) and a linear correlation (r=0 95, p<0 001) between GFR and TmP was found in all 15 patients (Fig 1)

To reduce the influence of GFR on TmP the TmP/GFR index was calculated This index varied over a wide range (0 03–0 85 μmol/ml mean 0 50) and when related to i PTH which also dispersed over a very wide range (1 4–13 0 ng/ml mean 4 0) a significant inverse correlation (r=−0 82 p<0 001) was found (Fig 2) This relation persisted when the influence of GFR was eliminated by use of a partial correlation coefficient Furthermore between TmP/GFR and i PTH inverse significant correlations were demonstrated in the group of transplanted patients (y=−5 44x+6 35 r=−0 95 p<0 001) as well as in the group of patients with chronic nephropathy (y=−12 53x+9 59 r=−0 82 p<0 05) Neither i PTH nor TmP/GFR showed any significant correlation to the serum calcium concentration or the extracellular volume The serum standard bicarbonate concentration was significantly correlated to the TmP/GFR index (p<0 05)

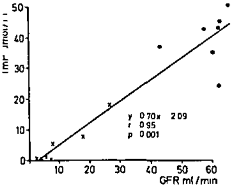


Fig 1 Correlation between GFR and TmP in 8 uremic (×) and 7 kidney transplanted (●) patients

but when related to i PTH no significance was obtained

Immunoreactive PTH concentrations were within the same levels in the two groups of patients ($p>0\ 05$) No correlation could be demonstrated between renal function and i PTH concentration ($p>0\ 05$) Serum phosphate concentrations estimated at the same time as i PTH did not differ in the two groups (mean 2 04 mmol/l) while serum calcium concentrations were slightly higher in the group of transplanted patients (mean 2 54 mmol/l) than in the group of patients with chronic nephropathy (mean 2 24 mmol/l) (Table I) A correlation between i PTH and serum calcium could not be demonstrated ($p>0\ 1$)

Finally the reproducibility of the estimation of TmP was calculated Nine of the 15 patients had the procedure repeated in another 120 min period immediately after the first one Our technique was found to be fairly reproducible with a coefficient of variation of 14%

DISCUSSION

The renal handling of phosphate depends on many factors apart from the parathyroid function (10) Primarily it depends on the renal function (9) but the acid-base balance (23) the calcium concentration (4) the extracellular volume (13) age (21) and the time of the day (17) are also important Some of these factors may mediate their influence through the parathyroid hormone Finally administration of vitamin D (19) and steroids (15 22) may affect the renal phosphate handling In the present investigation the TmP/GFR index was found to be inversely correlated to the serum concentration of i PTH in patients with a very wide range of TmP GFR and i PTH This correlation has not been demonstrated before

Our material included 6 uremic patients with secondary hyperparathyroidism as well as 4 kidney transplanted patients with clearances of approximately 60 ml/min and persisting hyperparathyroidism This material where i PTH levels did not correlate to the renal function may explain why a significant correlation was obtained between i PTH and the renal handling of phosphate when other investigators (8 18) using different materials (and techniques) have not been able to demonstrate this correlation

One cannot disregard the possibility that the correlation found in the present material was due to parallel changes in i PTH and TmP/GFR without any causal connection However of the mentioned factors known to influence the tubular reabsorption of phosphate the i PTH concentration in serum was the only one which could be significantly correlated to the TmP/GFR index apart from a statistically borderline correlation between the index and the standard bicarbonate concentration in serum the latter at least partly being regulated via the parathyroid function (14) None of the patients received vitamin D while 6 of the kidney transplanted patients received steroids (7 5–25 mg prednisone/day) which may alter the tubular reabsorption of phosphate It has been shown that this effect is not mediated via the parathyroid hormone (12) The TmP in these six patients (mean

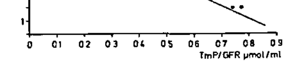


Fig 2 Correlation between TmP/GFR index and i PTH in 8 uremic (×) and 7 kidney transplanted (●) patients

39 μmol/min) did not differ from TmP of the transplanted patient (no 3) who did not receive prednisone (Table I) This may suggest that the administration of prednisone in our patients did not alter the renal phosphate handling significantly

Thus although radioimmunological PTH assays should always be interpreted with some caution especially in patients with renal failure (3 20) the present data indicate a key role of the parathyroid hormone in the regulation of phosphate excretion in patients with impaired renal function

This conclusion is in accordance with other investigators (1 7 24) who using techniques different from ours have demonstrated that the parathyroid hormone is the major determinant of renal phosphate handling in advancing renal disease

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The Functional Pattern of the Transplanted Kidney during the First Year

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ABSTRACT Prospective studies on kidney function have been carried out 1-14 months after transplantation in two groups of recipients of renal transplants groups were characterized by immediate graft The first group had transplants from living donors, the second had received cadaveric kidneys In the first group a functional maximum (as measured by [125]iothalamate, creatinine and [131]I hippuran clearances) was reached during the first three weeks after transplantation, approximating 70% of the donors' bilateral preoperative function In the second group kidney function was constant during the period of study, the values being almost identical with those observed in the first group At investigation 12-14 months after transplantation, the two groups were compared with the remaining cadaveric transplants carried out during the period of study, the latter being primarily graft anuric Graft function in the primarily anuric grafts was found to be poorer than in those with initial function Graft survival too, was poorer at one year in the group characterized by primary graft anuria than in the other groups Graft survival at one year was 68% in cadaveric kidney transplants with good initial function

The outcome of a renal transplantation varies according to whether the graft originates from a living donor or is cadaveric Graft survival at one year posttransplant is 75-80% for living donor grafts and 50-55% for cadaveric kidneys (2 4 13) The most important reasons for the poorer results with cadaveric kidneys are poorer histocompatibility and longer ischemia times

The present work involves a prospective determination of function in two groups of recipients after renal transplantation and a comparison of graft function and graft survival in these two groups with a third group 12-14 months after transplantation In the prospective study the two groups had received grafts from living and deceased donors respectively Both groups were characterized by immediate graft function with creatinine clearances of more than 8 ml/min during the first 24 hours after transplantation The cadaveric kidney recipients were thus a selected group since only 27% of such transplants performed during the period of investigation achieved this level of renal function At the study 12-14 months after transplantation the group with good initial function of cadaveric transplants was compared with the other cadaveric recipients from the same period the latter group was characterized by primary graft anuria

The purpose of the present study was to investigate whether functional differences during the first year after transplantation could be demonstrated in the various groups of transplant recipients In addition an attempt was made to define which factors in posttransplant treatment influence graft function and graft survival

MATERIAL

The primary study material comprised 106 transplants of which 39 were included in the prospective study and 67 in the comparative study undertaken 12-14 months after transplantation The latter study also includes the results of function studies in 11 donors these being compared with the recipients function after transplantation (Table III)

Group I Living donor transplants (Tables I and II) The group consisted originally of 14 recipients of a kidney from a living donor (5) Two grafts were destroyed during the first 6 days after transplantation because of severe acute allograft reactions In one patient graft function subsided during the course of 3 months because of a severe chronic allograft reaction Remaining at follow up were 11 patients

Table I *Kidney function studies in living donor transplants (group I) 1–3 and 3–12 months after transplantation*

C_{Io} = ¹²⁵I iothalamate clearance C_{Cr} = creatinine clearance C_{131I-h} = ¹³¹I hippuran clearance FF = filtration fraction
1 = 1–3 months 2 = 3–12 months after transplantation

T no	Age (y)	Proteinuria (g/24 h)		Blood pressure		C_{Io} (ml/min)		C_{Cr} (ml/min)		C_{131I-h} (ml/min)		FF C_{Io}/C_{131I-h}
		1	2	1	2	1	2	1	2	1	2	1
74	14	0	0	120/89	125/80	55	60	63	71	239	238	22 9
76	46	0	0	150/95	180/100	63	71	72	91	315	304	20 0
77	41	0	0	155/100	170/95	43	43	50	86	257	269	16 7
78	45	0	0	145/95	190/100	97	80	96	93	403	331	24 1
79	47	0	0	175/115	140/90	42	44	54	75	239	195	17 6
81	48	0	0	165/100	160/100	68	64	86	85	297	278	22 9
84	53	0	0	145/90	155/100	60	65	89	105	280	226	23 2
86	36	0	0 4	140/90	165/90	66	58	100	109	429	363	15 4
91	23	0	0	135/100	135/100	79	79	102	116	273	313	28 9
95	14	0	0	130/80	140/105	26	31	33	46	124	135	21 0
100	23	0	0	120/80	120/85	82	54	112	88	384	265	21 4
Average	35			144/95	153/95	62	59	78	88	295	265	21 3
S D	14			17/11	22/8	20	15	25	19	87	65	3 8
S E M	4 3			5/3	7/2	6 1	4 6	7 6	5 9	26	20	1.2

aged 15–54 years three women and eight men All had stable graft function during the period of study and four were being treated for hypertension

Groups II and III recipients of cadaveric transplants Altogether 84 recipients who had received 92 cadaveric kidneys during the period April 1969–June 1971 (11)

Group II (Tables III IV and VI) The group consisted nally of 24 recipients of 25 grafts (11) in whom graft in terms of creatinine clearance was more than 8 ml/min during the first 24 hours after transplantation Three grafts in two patients were destroyed during the first 20 days because of severe allograft reactions patients died during the study period three becau septicemia and one because of heart failure in conne with a severe acute rejection accompanied by throm of the graft artery the fifth died of a pulmonary embo the graft showing severe chronic allograft reactio further patient (T 140) was excluded from the prospe study because of ureteral necrosis leading to perm cutaneous ureterostomy but with good renal function remaining 16 recipients comprised eight women and men aged 29–60 years All had stable graft function d

Table II *Kidney function studies in living donor transplants (group I) 12–14 months after transplant related to donors pre and postoperative function*

R = recipient function 12–14 months after transplantation D_1 = donor function before D_2 = 32–40 months after unil nephrectomy other abbreviations as in Table I

T no	Age (y)	Proteinuria (g/24 h)	BP	C_{Io} (ml/min)			C_{Cr} (ml/min)			C_{131I-h} (ml/min)			FF C_{Io}/C_{131I-h}	
				R	D_1	D_2	R	D_1	D_2	R	D_1	D_2	R	D_1
74	15	1 6	150/100	40	89		62	102		153	437		26 1	20 9
76	47	0	170/95	61	94	69	91	108	73	244	411	249	25 0	22.8
77	42	0	135/80	51	83	67	71	107	58	199	343	212	25 6	24 2
78	46	0	190/100	45	113	78	58	135	91	209	402	232	21 5	28 0
79	48	0 5	150/95	35	82	97	77	134	95	153	650	352	22 9	12.6
81	48	0	160/90	70	101	89	101	88	94	289	633	413	24 2	15 9
84	54	0	140/95	71	119		103	105		263	401		27 0	27 2
86	37	0 4	145/100	40	128	96	98	118	98	268	492	358	14 9	26 0
91	23	0	140/90	76	104	94	97	102	95	319	489	318	23 8	21.3
95	15	0	130/90	37	102	87	50	135	89	139	523	310	26 6	19.5
100	24	0	120/80	71	100		134	106		336	501		21 1	20 0
Average	36		148/92	54	101	84	86	112	87	233	480	305	23 5	21 7
S D	14		20/7	16	14	12	25	16	14	68	96	70	3 5	4 7
S E M			6/2	4 8	4 4	4 2	7 4	4 7	4 9	21	29	25	1 0	1 4

ı e III *Kidney function studies in cadaveric transplants (group II) 1–3 and 12–14 months after trans ›lantation*

ˬviations as in Tables I and II

	Age	Proteinuria (g/24 h)		Blood pressure		C_{Io} (ml/min)		C_{Cr} (ml/min)		$C_{131I\,h}$ (ml/min)		FF $C_{Io}/C_{131I\,h}$	
Γ no	(y)	1	2	1	2	1	2	1	2	1	2	1	2
75	37	0 5	0	135/90	110/75	103	99	93	133	373	399	27 6	24 8
83	44	0 6	0	130/100	160/95	51	55	57	85	273	271	18 7	21 9
96	60	0	0	105/80	165/100	48	65	88	91	290	335	16 6	19 4
101	49	0	0	145/90	150/100	45	71	74	100	215	256	20 9	27 7
))	44	0 3	0	100/50	135/85	89	57	107	71	316	193	28 2	29 5
105	29	0	0	155/100	140/105	82	80	74	106	425	408	19 3	19 6
111	50	0	0	130/90	160/110	56	70	60	101	235	251	23 8	27 9
112	47	0	0 3	125/90	130/90	32	29	62	49	155	96	20 6	30 2
118	51	0	0	160/90	145/100	72	84	91	106	287	390	25 1	21 5
137	58	5 2	10 6	200/105	170/95	68	72	80	101	290	249	23 5	28 7
149	43	0	0	160/90	175/100	50	88	64	84	230	255	21 5	34 4
154	48	0	0	130/100	140/100	40	76	65	92	233	305	17 2	24 9
156	23	0	0	130/80	130/90	56	59	64	69	306	220	18 3	26 8
161	60	1 7	0	160/105	145/100	42	42	65	53	214	221	19 6	19 0
172	47	0	0	165/100	180/120	41	41	55	63	163	155	25 2	26 5
178	54	0	0	150/80	140/70	77	63	76	77	344	304	22 4	20 7
Average	47			143/91	148/96	60	66	73	86	271	271	21 8	25 2
S D	10			25/14	19/12	20	18	15	22	73	89	3 7	4 5
S E M				6/4	5/3	5 0	4 6	3 7	5 5	18	23	0 9	1 3

period of study Five had hypertension and one was on antihypertensive treatment

Group III comprised 60 recipients of cadaveric trans plants seven of whom had received two transplants dur ing the period of study 31 women and 29 men age 18–59 years All evidenced initial anuria at transplantation or creatinine clearance values of less than 8 ml/min Addi tional data are given in Table IV

Histocompatibility expressed as worst possible match (15) is given in Table VI

Perfusion was undertaken in most cases with Perfudex® Mechanical perfusion was not used

Immunosuppressive therapy consisted of azathioprine 2 5 mg/kg/day and prednisone which during the period of study was reduced from 40 mg/day to 10–15 mg/day All patients underwent bilateral nephrectomy during the period of study

METHODS

In the prospective study simultaneous ^{125}I-iothalamate creatinine and ^{131}I hippuran clearances were performed using a technique described earlier (5 9 11) Three studies were performed during posttransplant months 1–3 3–12 and 12–14 Comparisons were made of function and the functional pattern between recipients of kidneys from living donors and cadavers respectively The comparison of function between groups II and III 12–14 months after transplantation was made with 12 and 24 hour endogenous creatinine clearances The relationship between graft survival age of the donor and ischemia times was investi gated At the time of the last study graft function in patients with kidneys from living donors was related to renal function in the donors

In calculating *p* values Spearman s test and Wilcoxon s rank sum test were used

Table IV *Data on groups II and III immediately posttransplant and on still functioning grafts 12–14 months after transplantation (mean+S D)*

	At transplantation					12–14 months after transplantation				
	No of grafts	Donor age (y)	Ischemia times (min) Warm	Cold	Recipient age (y)	No of grafts	Donor age (y)	Ischemia times (min) Warm	Cold	Recipient age (y)
Group II	25	32±14	27 9±22 4	351±181	45±11	17	33±15	25 6±19 5	301±166	46±10
Group III	67	43±16	27 4±16 8	427±192	42±11	31	40±15	28 1±18 4	410±205	41±10

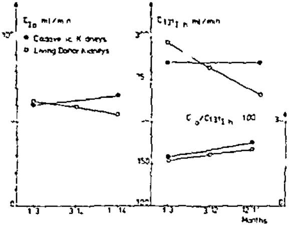


Fig 1 [125]Iothalamate (C_{Io}) and [131]I hippuran clearance ($C_{131\,I\,h}$) values and filtration fraction ($C_{Io}/C_{131\,I\,h}$) in the recipients of cadaveric and living donor kidney transplants with good initial graft function

RESULTS

In group I (Tables I and II and Fig 1) glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) showed a gradual non significant fall in the three periods 1–14 months after transplantation In group II values for GFR and ERPF hardly changed between the three studies The results from the first and the last study are given in Table III and Fig 1 Values for filtration fraction (FF) did not deviate significantly from corresponding values in normals (10)

At the study 12–14 months after transplantation graft function in the recipients of kidneys from living donors (group I) was compared with renal function in donors before and after unilateral nephrectomy Graft function in the recipients amounted on an average to 53 77 and 49% respectively of the donors bilateral [125]iothalamate creatinine and [131]I hippuran clearances Eight donors were studied 32–40 months after unilateral nephrectomy Graft function then averaged 60 92 and 75% of the donors remaining renal function in terms of [125]iothalamate creatinine and [131]I hippuran clearances respectively There was no correlation bet values for graft clearance and donor clearan fore and after unilateral nephrectomy (Table I

Comparing groups I and II 12–14 months transplant showed no significant differenc GFR ERPF and FF (Fig 1) In group I there three patients with slight proteinuria while on tient in group II had a proteinuria of 0 3 g/24 l and another a massive proteinuria of 10 6 hours The average blood pressure was simi the two groups Four patients in both groups treated with antihypertensive drugs Averag was significantly higher in group II than in gr Graft survival in group I was 79% while in gr it was 68% The survival figure for the latter includes one patient (T 140) with a well functic graft who had to be eliminated from the fun study because of a permanent cutaneous ur ostomy

A comparison between groups II and III l months after transplantation demonstrated a kedly poorer graft survival in group III tha group II the former being 46% Qualitatively function was poorer in the remaining grafts in g III than in group II the 12 and 24 hour endoge creatinine clearances being 76 and 58 ml/min spectively (the difference is significant $p<$ (Table V) Histocompatibility studies gave highest matching score in group I The percen of A matches in group II was higher than in g III (Table VI) Donors to recipients in grou were older than those in group II though the ference was not significant Neither were significant differences in recipient age or warm cold ischemia times between the two groups A age values are given in Table IV The ave blood pressure level was the same in groups II III while patients in group III averaged a lo period of dialysis before transplantation than tl in group II (Table IV)

Table V *Data on recipients of cadaveric kidneys 12–14 months after transplantation*

	N	12 or 24-hour creatinine clearance (ml/min)			Blood pressure (mmHg)			Age (y)	Averag no of dialyse
		Average	S D	S E M	Average	S D	S E M		
Group II	17	76	21	5 1	148/94	18/13	4 4/3 3	46	38
Group III	31	58	21	3 8	146/93	19/11	3 5/1 1	41	70

VI Histocompatibility (no of worst possible matches—A C D—) in the three groups at transplantation and in grafts surviving 12–14 months

	At transplantation				12–14 months after transplantation			
	A	C	D	Total	A	C	D	Total
Group I	7	6	1	14	6	5	0	11
Group II	4	10	11	25	3	7	7	17
Group III	4	32	31	67	1	15	15	31

DISCUSSION

In the two groups of patients with a well functioning transplant renal function studies were undertaken during the first 14 months after transplantation Both groups were characterized by graft function immediately after transplantation Group I comprised patients with transplants from living donors Group II had cadaveric transplants and was selected from a series of 92 consecutive transplantations The remaining larger part of this group characterized by no or only slight graft function comprised group III

Recipients of kidneys from living donors reached a maximal graft function amounting to 70% of the donors preoperative bilateral values during the first three posttransplant weeks (5) Since 50% of donors total renal mass had been removed (10) this means that an ability for compensatory hypertrophy is retained in the transplanted deinnervated kidney During the course of the first year graft function diminished amounting 12–14 months after transplantation to 50% of the donors preoperative values in terms of ^{125}iothalamate and ^{131}I hippuran clearances This is at variance with previous studies (3) in which a maximum graft function of about 60% of the donors bilateral values was not reached until six months posttransplant Creatinine clearances were constant throughout the course and comprised 75% of donor values Percentage differences between creatinine and ^{125}iothalamate clearances increased during the period of study A similar difference between ^{125}iothalamate clearance and creatinine clearance was observed in patients with the nephrotic syndrome chronic glomerular nephritis and interstitial nephritis (12) In cadaveric graft recipients ^{131}I hippuran clearance was constant from the 20th day after transplantation whereas ^{125}iothalamate clearance increased slightly (11) At the end of the period graft function was almost identical in the two groups despite a much better histocompatibility for the HL A system in group I and in spite of longer ischemia times in group II

A number of factors influence graft survival Differences in histocompatibility and the quality of the donor kidney are given as the most important (1 6 7 8 13 14) This is shown by longer graft survival times in recipients of kidneys from closely related living donors than in recipients of cadaveric kidneys (4 6 13) The one year survival times in the present material of kidneys from living donors (group I) and cadaveric kidneys (groups II and III) were identical with those in much larger groups that is 78 and 53% respectively reported elsewhere (2 4)

One year after transplantation there was no difference in graft function between groups I and II which are both characterized by good initial graft function Graft survival was poorer in group II than in group I 68 against 78% Between groups II and III there was a significant difference in graft function measured by means of 12 or 24-hour endogenous creatinine clearance With regard to graft survival the difference was likewise greatest between these two groups 68 and 46% respectively The two groups were identical with regard to ischemia times (Table IV) and histocompatibility (Table VI) neither did the age of the donors differ significantly (Table IV) The only difference between groups II and III was that graft function was initially good in group II This study has thus shown that a good initial graft function is of great importance for later graft function

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Psychological and Social Problems Encountered in Active Treatment of Chronic Uraemia

II *The Living Donor*

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ABSTRACT Sixty four kidney donors have been interviewed by a psychiatrist and given the Rorschach test 6 months–6 years after the transplantation Twenty three of these donors were also interviewed before the operation In addition, each case was studied in detail at a case conference On the basis of the data thus obtained, an analysis was made of the central dynamics of the donors personalities, of how they had experienced the donation and of how they had adapted to it No psychic trauma was observed in 20 of the subjects and in 5 of these the operation had apparently had a beneficial effect on their psychic well being That no trauma was observed in 8 additional donors was due largely to the effects of a multitude of other traumatic life experiences Mild trauma had been experienced by 24 donors and moderate to severe by 12 In our study the donation of a kidney to a sibling turned out to be more traumatic than the donation to a child, and a transplantation with an unsuccessful outcome was more often associated with psychic trauma to the donor than was a donation with a successful outcome Psycho-social factors that substantially lessened the likelihood of trauma were good inner resources, flexible defence mechanisms, good mental health and mild compulsive traits Factors that favoured traumatization were poor living conditions interpersonal problems limited inner resources, low self-esteem (narcissistic problems) and severe psychic deviancy The results clearly show 1) that potential donors ought to be given adequate time not only to consider their decision but also to prepare themselves for the actual donation, and 2) that supportive help should be offered both before and after transplantation, to donors whose psycho-social profiles reveal them to be vulnerable to traumatization

Kidneys for renal allografts are provided by cadaveric donors or by living donors usually close relatives There is no doubt that a well matched transplant from a related living donor offers much better results as regards patient survival function of the kidney and severity of the rejection process than does a cadaver transplant (4) However although unilateral nephrectomy does not harm the donor physically (2 5) the giving up of a kidney to a relative is an experience often fraught with emotional distress that is usually related to the spontaneity and the maturity of the decision We therefore studied the nature and the incidence of the emotional problems that living donors experienced which could be attributed to their participation in the transplantation procedure

SUBJECTS

The study comprised 64 of the 67 persons who voluntarily donated kidneys for the series of renal transplantations performed during 1966–72 in the University Central Hospital Helsinki Among the relatives who spontaneously volunteered to be donors that relative had been chosen whose histocompatibility testing and medical examination proved to be the most suitable Three of the living donors were excluded from the study due to practical inconveniences such as exceedingly long distances Of the 64 donors studied 38 had donated their kidney to a sibling and 26 to a child The latter group included one donor who had given up her kidney to a grandchild

The control group consisted of 10 patients on whom a unilateral nephrectomy had been performed for an illness of their own

METHODS

All the subjects were interviewed and given the Rorschach test by the team psychologist 6 months–6 years after the

Table I *Level of trauma of donor classified according to type of recipient*

Donor	Recipient	
	Child	Sibling
No trauma		
Not present	11	9
Not discernible	3	5
Mild trauma	8	16
Moderate to severe trauma	4	6

transplantation A follow up was usually made after more than one year Twenty three subjects were also interviewed before the operation Each case was then analyzed at a case conference Special attention was paid to the central dynamics of the subjects personalities to how they had experienced the donation and how they had adapted to it From the information assembled at the case conference a short clinical vignette was prepared on each subject

RESULTS

Few donors spoke spontaneously about the donation it turned out to be a sensitive topic and seemed often to evoke jealousy conflicts in the family The husband of one prospective donor for example *would not have allowed his wife to donate a kidney* to a member of her family

The experience of the donation and the adaptation to it varied considerably from subject to subject For many subjects the donation was a psychically traumatic experience The term *traumatic* is used here in its usual psychoanalytic and psychiatric meaning *An experience is considered to be* traumatic when because of the intensity and/or abundance of stimuli the capacity to handle the stimuli is exceeded This in turn provokes anxiety and regression as well as a compulsion to repeat the experience in one s mind in order to gain belated control over it to reduce tension or both (3)

The donors were classified according to the extent to which the event actively persisted in their minds producing ideational material related to it i e according to how traumatically they had experienced the donation This resulted in three groups 1) no trauma $n=28$ (not present in 20 not discernible in 8) 2) mild trauma $n=24$ 3) moderate to severe trauma $n=12$ The subject was regarded as not having suffered a trauma if a) he or she had not experienced the transplantation traumatically maintaining adequate emotional control over the event (*5 subjects had in fact clearly profited psychically from the operation in that the donation of kidney had eased intrapsychic or interper... problems*) or b) a possible trauma could n... discerned either because of the general per... pathology or the massive influence of other ... *matic circumstances*

Table II *Level of trauma of donor classif... according to outcome of transplantation*

Donor	Outcome of tr... plantation	
	Successful	Un... ces...
No trauma		
Not present	15	5
Not discernible	6	2
Mild trauma	16	8
Moderate to severe trauma	1	5

As many control patients as donors in re... terms experienced the loss of a kidney as ... matic mildly traumatic or moderately to se... traumatic Thus for some of the control pa... as well the operation remained a continousl... turbing inner reality

Correlating the level of trauma experience... the donor with the type of recipient (sibling or ...) gave the results presented in Table I The d... ences between the three groups of donors we... statistically significant

Table III *Dynamically important psychos... factors in different levels of trauma experienc... donors*

Level of trauma	
No trauma	
Not present	Good inner resources flexible defence mechanis... good mental health mild compulsive traits
Not discernible	Extreme defensiveness vulnerable psychic balance abundance of other trauma
Mild trauma	Problems with aggression depressive problems
Moderate to severe trauma	Poor living conditions interpersonal problems limited inner resources low self-esteem (narcissistic problems) severe psychic demancy

The level of trauma experienced by the donor , then correlated with the outcome of the trans ıntation (Table II) The transplantation was re as successful if the recipient no longer f regular dialysis treatment Again there was statistically significant difference between the groups

A scrutiny of the clinical vignettes of the subjects ealed that certain key factors primarily person ity characteristics had influenced the reaction of three donor groups as classified by level of ıma toward the transplantation (Table III)

An evaluation of the 23 subjects who had been jected to psychiatric consultation before and af the transplantation revealed that in none had change in their basic personality taken place ring this interval

DISCUSSION

he kidney donors were reluctant to talk about the splantation They hardly even mentioned it spon y while talking with the team psychologist 1 the psychiatric interview most subjects em hasized that they rarely or never discussed this atter with relatives friends or acquaintances ney rationalized this reticence in various ways , did not want to boast about the donation they ıd not want to provoke feelings of guilt in the ecipient their attitude was simply one of that s hing to talk about and so on These explana ons must however be regarded as secondary Evi ence from the tests and interviews indicated that explanations stemmed from the idea that the onation was a painful sacrifice and the source of ch anxiety This agrees with the observation of (1) that the donation of a kidney is experi ced primarily as mutilating and hence is ac by anxiety and in various degrees by a ling of having been damaged

The similarity in the grouping of control patients nd donors by the level of trauma experienced oints to the importance of experiential factors in ch an operation We believe therefore that an nterpretation of the results of a study such as this ocus on the personality of the subject and on the mutilating nature of the operation In other vords the problem of adaptation after a major sur cal operation is a product of the covariance of factors Among these are many psychologi and social psychological ones that patients recovering from all types of surgery may have in common regardless of whether their motives for the surgery appear to be egoistic or altruistic

Twenty subjects had apparently not been trau matized at all by the donation The good inner resources and flexible defenses of these persons rendered them able not only to absorb external emotional impacts but also to bind relatively large amounts of anxiety Their fundamentally high self esteem afforded them the possibility of compensat ing for the feelings engendered by the inflicted de fect

In the five instances in which the donation had had a beneficial effect on the psychic well being of the donors two dynamic factors seemed to be operating firstly the feeling of having been able to help one s suffering relative and secondly a reduc tion in the feelings of guilt caused for instance by being in good health while one s close relative was in constant danger of death For the five people in question these factors provided adequate compen sation for the emotional risks involved in the donor operation

The emotional impact of the donation on the eight subjects in whom trauma was not discernible was undoubtedly greater than that revealed by the tests and interviews but the true extent of the trauma remained undiscernible largely because of the multitude of traumatic life experiences of these persons

Twelve subjects were moderately to severely traumatized by the donation of a kidney and by the events that followed Their limited capacity for binding anxiety caused the anxiety provoked by the donation to become overt and poorly controlled Their easily vulnerable self-esteem rendered them incapable of compensating adequately for the distressing emotions that the operation evoked When the transplantation for which she had donated a kidney was unsuccessful one unmarried female donor previously treated for a brief state of confu sion became psychotic and had paranoid symp toms for a long period

In several cases jealousy conflicts presented a distinct group of problems These conflicts can at least partly be understood as an expression of a disturbed narcissistic balance within the family The spouse of the donor experienced his or her partner as having placed the family in a more vul nerable position to the benefit of someone not be longing to the nuclear family as though an inner

patients had a parathyroid adenoma which was necessary to remove

Gastrointestinal complications such as moderate liver damage, often due to the immunosuppressive treatment, sometimes occur (3) Hepatitis may of course be present in some cases but was not found in our series Ulcers also occur during the later course mainly as a result of the continuous corticosteroid therapy One of our patients died of a perforating ulcer As previously reported gastrointestinal complications are common and often dangerous following a renal transplantation (2) The incidence of infection is very high during the first few postoperative months but an infection may also develop later Our series comprises one case of Candida infection of the lung and two cases of herpes zoster Haematological complications in the form of leucopenia are more common during the early course Later the bone marrow may react resulting in erythraemia as in two of our cases

Serious psychic complications such as depression neuroses or psychoses may occur It is sometimes necessary to reduce the cortisone dose to a minimum The present series includes a case of attempted suicide Miscellaneous complications were represented by a case of cataract It deserves mentioning that no case of secondary renal arterial ste is nor any malignant tumours were observed

All degrees of histocompatibility were represented in the series The age and sex incidences as well as the distribution by basic disease were similar to those in other transplantation series Hence the occurrence of complications cannot be correlated to any of the above mentioned parameters

It thus appears that both mild and severe complications may occur during the late course of a transplantation although the incidence is relatively low By way of conclusion it may be stated that if the course is favourable during the first six months there is a good chance of a more or less uneventful continuation although even severe complications occur occasionally

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Iron Absorption after Renal Transplantation

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ABSTRACT Gastrointestinal iron absorption has measured by means of whole body counting in 13 patients after renal allotransplantation Whole retention 14 days after oral administration of μCi ^{59}Fe together with a carrier dose of 9 9 mg was used as an expression of absorption The incorporation in the total erythrocyte of administered ^{59}Fe (erythrocyte incorpora and absorbed ^{59}Fe (red cell utilization) was as well Geometric mean iron absorption 12 4±2 5 (S D) % and geometric mean erythro- rporation 11 1±3 0 (S D) % while arithme mean red cell utilization was 95 6±8 6 (S E M) None of these parameters differed significantly those obtained in normal subjects (p>0 2 >0 1, p>0 3, respectively) Iron absorption and throcyte incorporation in renal transplanted pa did not differ significantly from the values in non dialysed and dialysed patients with c renal failure (p>0 1) The correlation be tween iron absorption and erythrocyte incorporation highly significant (r=0 96, p<0 001)

A successful renal allotransplantation (RAT) is followed by a distinct regression of the anemia encountered during the pretransplant uremic stage (3 8 12 15 19) primarily through an augmented production of renal erythropoietic stimulating factor (REF) (1 5 19) inducing an increase in the erythrocyte volume and the Hb mass

Our previous investigations have demonstrated that the iron absorption capacity is normal in non dialysed patients with chronic renal failure and in patients undergoing regular hemodialysis (17 18) However the few data available on renal transplanted patients indicate a diminished absorption and these reports (3) prompted the present study with the purpose of evaluating gastrointestinal iron absorption after renal transplantation

CASE MATERIAL

Thirteen patients (6 males 7 females) participated in the study (Table I) All had been transplanted with cadaver renal allografts 3–50 months before the investigation and had stable kidney (graft) function with serum creatinine within the normal range and 24 hour endogenous creatinine clearance ≥50 ml/min They were on a normal diet and antacids and anticholinergic drugs were administered by routine Immunosuppressive therapy consisted of prednisone (5–30 mg daily) and azathioprine (25–175 mg daily) None of the patients were infected had been subjected to gastrointestinal surgery or had clinical signs of malassimilation Coombs tests were negative and serum bilirubin was normal

The control group consisted of 27 normal subjects (8 males 19 females) Details concerning this group have been published previously (14)

METHODS

Iron absorption was measured by whole body counting (14) Measurements of the whole body ^{59}Fe activity were performed at 4 hours and 14 days after the oral administration of 10 μCi ^{59}Fe together with 9 9 mg Fe^{2+} (as sulphate) as carrier dose to the fasting subject and corrected for background and radioactive decay before calculation of the percentage absorption of ^{59}Fe Erythrocyte volume was estimated by the method described by Jarnum (11) and the whole blood activity of ^{59}Fe was measured at the last counting procedure in order to calculate the erythrocyte incorporation i e the percentage of administered ^{59}Fe recovered in the total erythrocyte mass and red cell utilization i e the percentage of absorbed ^{59}Fe recovered in the erythrocytes

Hematological and biochemical parameters were measured by procedures described earlier (14 17) and reticulocyte counts were corrected for variations in hematocrit (22) Bone marrow specimens were obtained by iliac crest puncture stained for iron with Prussian blue and the iron content was graded according to Rath and Finch (20)

Logarithmic transformation of the measured values for iron absorption and erythrocyte incorporation was performed in order to obtain a normal distribution (4) In statistical analysis regression lines were calculated by the

Table I. *Clinical, renal and hematological data on 13 patients investigated for iron absorption after transplantation*

Pat no	Sex	Age (y)	Serum creatinine (mmol/l)	Creatinine clearance (ml/min)	Serum B_{12} (pmol/l)	Erythrocyte folate (nmol/l)	Erythrocyte glucose-6-phosphate dehydrogenase (U/mean erythrocyte)
1	♂	60	0.09	65	485	671	221
2	♂	33	0.09	90	485	326	227
3	♂	52	0.09	65	474	354	280
4	♂	25	0.12	100	618	332	279
5	♂	34	0.11	75	292	648	363
6	♂	43	0.12	50	466	490	232
7	♀	23	0.09	65	751	485	326
8	♀	32	0.12	55	548	392	272
9	♀	23	0.09	65	400	462	292
10	♀	34	0.11	63	340	697	268
11	♀	20	0.11	55	681	463	192
12	♀	31	0.10	50	640	290	245
13	♀	51	0.07	55	–	666	226
Arithmetic mean		36	0.10	66	515	481	263
S.D.		13	0.02	15	138	146	46
Normal range			0.04–0.13	96–120	140–600	247–665	205–320

method of least squares and the Mann Whitney rank sum test was used to evaluate significance of differences between patients and normal subjects.

RESULTS

Measurements of iron absorption and erythrocyte incorporation together with clinical, hematological and biochemical data are summarized in Fig. 1 and Tables I and II.

Iron absorption and erythrocyte incorporation of ^{59}Fe

Renal transplanted patients had an arithmetic mean iron absorption of 18.1±15.9 (S.D.) % (Fig. 1) and a geometric mean absorption of 12.4±2.5 (S.D.) %. The geometric mean erythrocyte incorporation was 11.1±3.0 (S.D.) % and the arithmetic mean red cell utilization 95.6±8.6 (S.E.M.) %. The present results are compared with values obtained in our previous studies of normal subjects, non-dialysed patients with chronic renal failure and patients in regular hemodialysis, as shown in Table III.

The mean iron absorption of the present patients was slightly higher than that of the normal subjects and the non-dialysed patients with chronic renal failure but the differences were not significant ($p>0.2$ and $p>1$, respectively). Also mean iron absorption was slightly, but not significantly, lower than in the patients on regular hemodi[alysis] ($p>0.1$).

The mean erythrocyte incorporation was slightly higher than in the normal subjects and the non-dialysed patients and lower than in the dialysis patients, but again the differences were insignificant ($p>0.1$, $p>0.1$ and $p>0.1$, respectively).

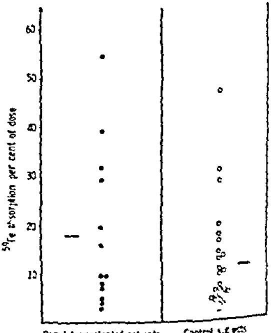


Fig. 1. Absorption of ^{59}Fe in renal transplanted patients and control subjects (arithmetic mean indicated).

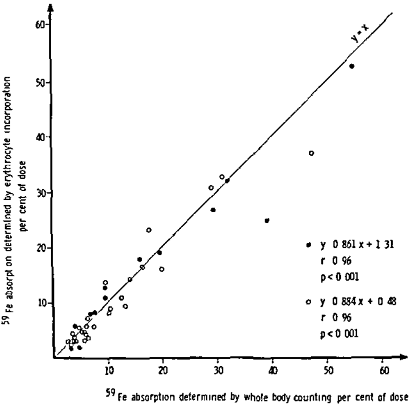


Fig 2 Relation between iron absorption and erythrocyte incorporation of ^{59}Fe in renal transplanted patients (●) and control subjects (O)

The red cell utilization of absorbed ^{59}Fe was higher in the renal transplanted patients than in the normal subjects the non dialysed patients and the dialysed patients but the differences were not significant ($p>0\ 3$ $p>0\ 05$ and $p>0\ 05$ respectively)

The correlations between iron absorption and erythrocyte incorporation were highly significant both in the patients ($r=0\ 96$ $p<0\ 001$) and the normal subjects ($r=0\ 96$ $p<0\ 001$) as demonstrated in Fig 2

Hematological studies

The Hb level was generally higher in the patients than in the controls but the difference was insignificant ($p>0\ 07$) One patient (no 5) presented with an iron deficiency anemia while the others had Hb values within the normal range The mean corpuscular volume was significantly larger in the patients than in the controls ($p<0\ 02$) while no difference was demonstrable concerning the mean corpuscular Hb concentration ($p>0\ 2$) and the reticulocyte count ($p>0\ 1$)

Bone marrow examination revealed normoblastic erythropoiesis Stainable hemosiderin iron was present in small quantities in 5 patients and absent in 4 patients

Biochemical studies

Three patients had subnormal serum iron values transferrin saturation below 15% and reduced or absent stainable marrow iron indicative of iron deficiency which was latent in 2 patients (nos 6 and 11) and overt in one patient (no 5)

The serum B_{12} erythrocyte folate and erythrocyte glucose-6-phosphate dehydrogenase were all within the normal range

DISCUSSION

The kidneys occupy a central position in the regulation of the erythropoietic activity through the production of REF (6) A subject with normal kidney function responds to an anemic stimulus (e g phlebotomy) with an increased production of REF (19) but in chronic renal failure this pattern of reaction is changed as demonstrated by the low and often unmeasurable plasma REF levels in anemic uremic patients (1 2 5 7 19) A successful RAT is

Table II *Hematological data iron absorption and erythrocyte incorporation in 13 patients after renal transplantation*

MCHC = mean corpuscular Hb concentration MCV = mean corpuscular volume TIBC = total iron binding capacity

Pat no	Hb (mmol/l)	Hct	MCHC (mmol/l)	MCV (fl)	Serum iron (μmol/l)	Plasma transferrin (μmol/l)	TIBC (μmol/l)	Transferrin saturation (%)
1	11 4	0 52	21 2	91	17 4	27 9	55 8	31 2
2	10 1	0 45	21 8	87	22 5	36 9	73 8	30 5
3	11 3	0 53	20 9	84	25 5	31 8	63 6	40 1
4	9 8	0 47	21 5	93	32 7	40 9	81 8	40 0
5	6 4	0 35	18 8	67	4 6	46 5	93 0	4 9
6	9 0	0 45	20 8	92	8 9	31 0	62 0	14 4
7	9 8	0 40	21 8	110	19 0	37 3	74 6	25 5
8	8 0	0 35	22 0	95	13 4	25 6	51 2	26.2
9	9 8	0 44	21 2	106	15 5	30 6	61 2	25 3
10	7 9	0 35	21 9	93	21 4	28 0	56 0	38.2
11	8 3	0 42	21 1	99	9 8	35 8	71 6	13 7
12	8 5	0 43	21 0	102	18 8	32 5	65 0	28 9
13	9 0	0 44	20 7	97	12 0	27 9	55 8	21 5
Arithmetic mean	9 2	0 43	21 1	94	17 0	33 3	66 6	26 [illegible]
S D	1 4	0 06	0 8	11	7 6	6 0	11 9	10 6
Geometric mean								
S D								
Normal range	7 0–10 5	0 35–0 52	18 6–22 3	81–109	10 7–34 0	24 2–47 7	48 4–95 4	

* Larsen & Milman (14)

followed by a rise in plasma REF (1 5 12 19) and an increased rate of erythropoiesis with normaliza of Hb plasma iron clearance plasma iron er and red cell utilization of i v ^{59}Fe (8 12 The REF has no direct influence on iron absorption but stimulates absorption indirectly through an accelerated erythropoietic activity (16)

The results of the present study indicate that renal transplanted patients possess the same gastrointestinal iron absorption capacity as normal subjects Nor is iron absorption significantly different in renal transplanted patients and patients undergoing regular hemodialysis We were thus unable to confirm the results of Boddy et al (3) showing a significantly lower iron absorption in transplanted patients than in normal subjects

Considering the increased iron loss in RAT patients due to frequent blood sampling one would have expected a significantly higher iron absorption in the transplanted than in the normal subjects However the routine administration of antacids anticholinergics and other drugs have possibly depressed the absorption

The prerequisites of obtaining an identical iron absorption in renal transplanted patients and normal subjects are among other factors an identical male/female ratio identical medication and comparable iron status and iron loss in the two groups of subjects These premises are not entirely fulfilled in the present series however it has previously been demonstrated that there is no significant difference between the iron absorption in males and females when a test dose of 10 mg of iron is employed in the absorption study (14)

Table III *Summary of iron absorption results in the present and previous investigations*

Subjects	Absorption of ^{59}Fe* (%)	Erythrocyte incorporation of ^{59}Fe* (%)	Red cell utilization of absorbed ^{59}Fe^{b} (%)
Normal subjects (14)	8.5±2 1	7 7±2.2	92 9±4 0
Non-dialysed patients with chronic renal failure (17)	9 6±2 0	7 5±2 3	80 3±4 8
Patients in regular hemodialysis (18)	14 3±2 0	11 6±2 3	84 4±6 0
Renal transplanted patients (present study)	12 4±2 5	11 1±3 0	95 6±8 6

* Geometric mean±S D ^{b} Arithmetic mean±S E M

arrow on –4+)	Corrected reticulocyte count (1/1 000)	Absorption of ^{59}Fe (%)	Erythrocyte incorporation of ^{59}Fe (%)
	25	4 0	5.5
	4	9 5	10 9
	21	39 0	24.5
	14	19 5	18 8
	6	9 5	12 8
	16	54 3	52 3
	18	7 6	7 8
	26	15 9	17 6
	13	31 7	31 6
	8	3 1	1 3
	10	29 2	26 4
	1	4 7	1 8
	13	6 8	7 9
	13	18 1	16 9
	8	15 9	14 3
		12 4	11 1
		2 5	3 0
	<12	1 9–38 3*	1 6–37 0*

The discrepancy between the present results and those of Boddy et al (3) can be attributable to various factors With the test dose used by Boddy et al (5 mg Fe^{3+}) absorption is lower in males than in females (10) thus making the investigation more sensitive to differences in the male/female ratio in the two populations compared Furthermore the administration of ferric instead of ferro compounds makes the absorption highly sensitive to variations in the duodenal pH (9) and no information was given about the gastric acid secretion in the 8 patients studied half of whom had an iron absorption of less than 1 2% Also the extension of the absorption study to 21 days tends towards a lower absorption due to possible intervening blood loss

Of the three iron deficient patients in the present series two (nos 6 and 11) showed a high iron absorption while one (no 5) had an unexpected low absorption The latter patient who had no achlorhydria received peroral anticoagulation therapy and was probably iron deficient due to an occult gastrointestinal bleeding It is evident that other factors than the iron status have influenced the iron absorption in this patient

The azathioprine treatment did not appreciably affect the erythropoietic activity as judged by the normal Hb and red cell utilization of absorbed ^{59}Fe However the relative macrocytosis in the transplanted patients in the presence of normal serum B_{12} and erythrocyte folate values suggests an interference of azathioprine with the metabolism of vitamin B_1 and/or folic acid (13 21) Increased hemolysis has been reported after RAT (12) but seems unlikely in the present series considering the normal serum bilirubin reticulocyte count and erythrocyte glucose-6-phosphate dehydrogenase which is a good indicator of the erythrocyte age (23)

The measurement of the erythrocyte incorporation of ^{59}Fe was performed as a control of the iron absorption (14) and the highly significant correlation between these parameters (Fig 2) confirms the reliability of the whole body counting method in the assessment of iron absorption.

The increase in the Hb mass after RAT makes heavy demands on marrow iron supplies iron loss due to blood sampling and hampered food iron absorption caused by treatment with antacids and anticholinergics are factors which predispose to the development of iron deficiency Provided that the iron stores are loaded before RAT iron depletion hardly occurs after transplantation but with pretransplant empty stores latent or overt iron deficiency is more liable to develop

ACKNOWLEDGEMENTS

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Table II *Hematological data iron absorption and erythrocyte incorporation in 13 patients after re[illegible] transplantation*

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5	6 4	0 35	18 8	67	4 6	46 5	93 0	4 9
6	9 0	0 45	20 8	92	8 9	31 0	62 0	14 4
7	9 8	0 40	21 8	110	19 0	37 3	74 6	25 5
8	8 0	0 35	22 0	95	13 4	25 6	51 2	26 [illegible]
9	9 8	0 44	21 2	106	15 5	30 6	61 2	25 3
10	7 9	0 35	21 9	93	21 4	28 0	56 0	38 [illegible]
11	8 3	0 42	21 1	99	9 8	35 8	71 6	13 7
12	8 5	0 43	21 0	102	18 8	32 5	65 0	28 9
13	9 0	0 44	20 7	97	12 0	27 9	55 8	21 5
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S D	1 4	0 06	0 8	11	7 6	6 0	11 9	10 6
Geometric mean								
S D								
Normal range	7 0–10 5	0 35–0 52	18 6–22 3	81–109	10 7–34 0	24 2–47 7	48 4–95 4	

* Larsen & Milman (14)

followed by a rise in plasma REF (1 5 12 19) and an increased rate of erythropoiesis with normalization of Hb plasma iron clearance plasma iron turnover and red cell utilization of i v ^{59}Fe (8 12 19) The REF has no direct influence on iron absorption but stimulates absorption indirectly through an accelerated erythropoietic activity (16)

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The prerequisites of obtaining an identical iron absorption in renal transplanted patients and normal subjects are among other factors an identical male/female ratio identical medication and comparable iron status and iron loss in the two groups of subjects These premises are not entirely fulfilled in the present series however it has previously been demonstrated that there is no significant difference between the iron absorption in males and females when a test dose of 10 mg of iron is employed in the absorption study (14)

Table III Summary of iron absorption results in the present and previous investigations

Subjects	Absorption of ^{59}Fe* (%)	Erythrocyte incorporation of ^{59}Fe* (%)	Red cell utilization of absorbed ^{59}Fe# (%)
Normal subjects (14)	8 5±2 1	7 7±2 2	9[illegible] 9±4 0
Non-dialysed patients with chronic renal failure (17)	9 6±2 0	7 5±2 3	80 3±4 8
Patients in regular hemodialysis (18)	14 3±2 0	11 6±2 3	84 4±6 0
Renal transplanted patients (present study)	12 4±2.5	11 1±3 0	95 6±5 6

* Geometric mean±S D # Arithmetic mean±S E M

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Cytostatic Treatment of Glomerular Diseases

III A Double Blind Cross over Study of the Effect of Cyclophosphamide Report from a Copenhagen Study Group of Renal Diseases

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ABSTRACT Fifty patients with renal glomerular diseases entered a double blind cross over study on the effect of cyclophosphamide, 38 had received neither corticosteroids nor cytostatic drugs before joining the study Cyclophosphamide was given for 4 months in doses decreasing from 3 to 1 5 mg/kg b wt Cyclophosphamide caused a 46% decrease in the 24-hour excretion of urinary protein and a decrease in serum creatinine within the normal range Albumin transferrin and IgA in urine, as well as albumin clearance and the sieving coefficient of albumin, changed parallel to the total urinary protein The initial values of proteinuria and serum complement were of prognostic significance for the effect of cyclophosphamide on serum creatinine We were unable to demonstrate a prognostic significance for the variables clinical diagnosis, renal histology, arterial BP, initial values of serum creatinine and IgG, IgA and IgM in serum and urine ESR appeared to be the most reliable acute phase reactant No differences were found between the changes in renal histology during cyclophosphamide or placebo

Treatment with cytostatic drugs has been used in various types of renal glomerular disease The results from treatment of glomerulonephritis have not been encouraging (12) Booth and Aber (4) did not observe any effect of 12 month treatment with cyclophosphamide or azathioprine on proteinuria serum creatinine or renal histology in adults with proliferative glomerulonephritis A similar negative result was reported by the Medical Research Council Working Party (5) from a controlled study of azathioprine and prednisone in a group of patients with various forms of glomerulonephritis Donadio et al (6) evaluated cyclophosphamide treatment for one year in a controlled prospective study of 22 adult patients with clinical and histologically defined idiopathic membranous nephropathy They observed no effect of cyclophosphamide on proteinuria renal function or renal histology Neither did McIntosh et al (11) detect any advantage in the use of azathioprine and prednisone in glomerulonephritis On the other hand azathioprine and cyclophosphamide may improve lupus nephritis (14) In children cyclophosphamide may prolong the period of remission in steroid sensitive nephrotic syndrome with minimal changes (3 10 13)

The purpose of the present paper was to investigate in a double blind cross-over study whether cyclophosphamide given through 4 months to patients with previously untreated renal glomerular diseases could influence proteinuria and/or creatinine clearance versus normalization A number of other variables in the blood and the urine were also analysed in order to further elucidate changes in the disease activity changes in immunological functions and side effects It appeared from the study that the proteinuria and serum creatinine decreased during treatment with cyclophosphamide No differences were found between the changes in renal histology during cyclophosphamide or placebo

Table I *Initial values of serum creatinine concentrations and proteinuria in relation to cl... diagnosis*

	No of pats	Serum creatinine (mg/100 ml)		Proteinuria (g/24 h)	
		Median	Range	Median	Range
Connective tissue diseases	10	0 9	0 5–1 9	1 5	0 2– 5 0
Glomerulonephritis	37	1 4	0 4–3 3	2 7	0 2–?1 1
Idiopathic nephrotic syndrome	3	0 8	0 7–1 0	2 3	0 0– 4 [illegible]

MATERIAL AND METHODS

The patients admitted to the trial fulfilled all of the following criteria 1) Histological glomerular changes 2) At least one of the following signs (*a*) decreased glomerular filtration rate as measured by creatinine clearance (*b*) proteinuria (>200 mg/24 h) or (*c*) pathological urinary sediment on microscopy 3) Lack of remission during the first month of observation. This group comprised 38 previously untreated patients Twelve previously treated patients fulfilling the admission criteria were also included in the material 10 of them had a renal disease resistant to azathioprine and/or prednisone one had glucocorticoid-resistant nephrotic syndrome and one glucocorticoid-dependent nephrotic syndrome Eighteen patients were females and 32 males 16 patients were 0–29 years of age 17 patients 30–45 and 17 patients more than 45 years of age

The clinical diagnoses (Table I) were based on the *clinical findings as well as on the histological glomerular* changes In 10 patients the renal diseases were part of connective tissue diseases (systemic lupus erythematosus 3 Henoch Schönlein purpura 2 rheumatoid arthritis 1 rheumatic fever 1 unclassified collagen disease 3) Patients with the clinical diagnosis of glomerulonephritis had a primary renal disease with histological glomerular changes of the types described in Table II and all these patients had at least two of the signs 2*a* *b* and *c* given above Patients with idiopathic nephrotic syndrome had minimal glomerular changes proteinuria normal urinary sediment hypoalbuminaemia and oedema

Laboratory investigations

The glomerular filtration rate was estimated by mean [illegible] creatinine clearance and serum creatinine which [illegible] determined at 2 week intervals Examination of [illegible] sediment quantitative determination of the total [illegible] protein excretion and arterial BP measurement [illegible] performed at similar intervals as described previo[illegible] (1) The concentrations of albumin α_2-macroglob[illegible] transferrin IgG IgA and IgM were determined immuno[illegible] chemically (9) at 4 week intervals in serum and [illegible] Samples were stored at −22°C and only frozen [illegible] thawed once Urine specimens were not concentrated [illegible] the sensitivity of the method was increased by [illegible] up to 20 µl urine to the wells The glomerular permeab[illegible] index or sieving coefficient of albumin was calculated [illegible] the albumin clearance relative to 24-hour creatin[illegible] clearance C3 and C4 complement in serum were de[illegible] mined immunochemically (9) at 4 week intervals Si[illegible] larly the total haemolytic complement activity of se[illegible] was determined at 4 week intervals by the method [illegible] Kabat and Mayer (8)

During treatment the following blood analyses were [illegible] formed at one or two-week intervals Hb leu[illegible] platelets reticulocytes ESR and plasma fibrinog[illegible] Serum bilirubin alanin aminotransferase alkaline pho[illegible] phatase and prothrombin in serum were analysed [illegible] 4 week intervals AST ASH ANA WR cold aggl[illegible] Coombs test and haptoglobin in serum were determ[illegible] at the beginning and the end of the study

Table II *Initial values of serum creatinine concentrations and proteinuria in relation to type of histol... glomerular lesions*

	Type of glomerular lesion*	No of pats	Serum creatinine (mg/100 ml)		Proteinuria (g/24 h)	
			Median	Range	Median	Range
1	Minimal changes	1				
2	Exudative	1				
3	Proliferative	19	0 9	0 4–2 2	1 2	0 0– 9 [illegible]
4	Lobular	2				
5	Extracapillary	4				
6	Epimembranous	1				
7	Membranoproliferative	12	1 3	0 8–1 7	3 8	0 8–21 1
8	Segmental focal	3				
9	Unclassifiable	7				
10	Type 1 + 2 4 5 6 8 and 9	19	1 3	0 5–2 8	1 8	0 1–10 2

* A principal classification is described in a previous paper (1)

Table III *Comparison of changes in the renal state after treatment with cyclophosphamide and placebo in 31 patients not previously treated with prednisone or azathioprine available for evaluation (both periods of treatment accomplished)*

For patients within solid triangles the best result was noted during cyclophosphamide for those within hatched triangles during placebo

		Changes in the placebo period								
		11 patients receiving placebo in the first period				20 patients receiving cyclophosphamide in the first period				Normalized or improved during cyclophosphamide (total)
		Normalized	Improved	Unchanged	Deteriorated	Normalized	Improved	Unchanged	Deteriorated	
Changes in the cyclophosphamide period	Normalized	1	1	0	0	1	0	0	0	13
	Improved	0	1	1	0	0	1	5	2	
	Unchanged	0	0	4	2	0	1	7	2	
	Deteriorated	0	0	1	0	0	0	0	1	
Normalized or improved during placebo		3				3				

Trial procedure

The trial was designed as a double-blind cross-over study After admission to the trial the patients were randomly allocated to cyclophosphamide or placebo during the first 4 months of treatment Thereafter the alternative treatment was given during the last 4 months

Treatment

The doses of cyclophosphamide were the first 2 weeks 3 mg/kg b wt the next 4 weeks 2 mg and the last 10 weeks 1 5 mg/kg b wt Placebo was administered in a number of tablets corresponding to the dose of cyclophosphamide Patients who were on prednisone treatment at the start of the trial continued with prednisone in the same doses during the entire trial If prednisone treatment had been discontinued before the trial it was resumed and given in doses of 0 3 mg/kg b wt during trial In patients showing severe progression of the renal disease during the study the code was broken If the patient was receiving placebo this was replaced by cyclophosphamide

Assessment

Apart from the analysis of the different variables under study the renal state of the patients at the end of each period of treatment was classified as *normalized improved unchanged* or *deteriorated* according to changes in serum creatinine proteinuria and urinary sediment as described elsewhere (1)

Statistical methods

Variables forming time series were analysed by Friedman tests (two-way analyses of rank variance) The test was performed on differences between chronologically corresponding values from the cyclophosphamide period and the placebo period The analyses were applied on the total unrestricted material as well as on subgroups By a second step analysis of variance it was examined whether there were significant differences between these subgroups Furthermore the Spearman rank correlation test Wilcoxon signed rank test Kruskall Wallis test the χ^2 test and the sign test were used All statistical tests were performed at the 5% level of probability unless otherwise stated

RESULTS

Six of the 56 patients who entered the study were excluded for the following reasons rapid progression made unblinding necessary in three patients (in one of them placebo was replaced by cyclophosphamide the other two were put on haemodialysis) lack of co-operation in one patient technical errors in two

Thirty-eight patients had received neither prednisone nor azathioprine before the start of cyclophosphamide treatment Seven of them did not complete the second part of the trial due to technical errors The remaining 31 patients are evaluated in Table III from which it appears that treatment with cyclophosphamide caused a shift in the renal state towards normalization This was true whether cyclophosphamide was given in the first or the second period of treatment Thirteen (42%) of the 31 patients improved during cyclophosphamide compared with six (19%) during the placebo period Thirteen patients had a better result during cyclophosphamide while two had a better result during placebo The latter difference between cyclophosphamide and placebo is statistically significant at the level * (two-sided test)

Table IV *Statistically significant alterations in variables during the observation period*

Type of alteration	Observations	Variables showing the alteration[c]
Improvement during *cyclophosphamide* (characteristic pattern)	Differences between *corresponding values* in cyclophosphamide and placebo period[a]	24 hour excretion of protein and albumin urine *concentration of albumin and transferrin* albumin clearance and sieving coefficient serum concentrations of creatinine and IgM
Improvement during cyclophosphamide	Relative increase from initial to final value[b]	24 hour excretion of protein (−46%) albumin (−34%) transferrin (−26%) and IgA (−42%) serum concentrations of albumin (+13%) transferrin (+10%) haptoglobin (−24%) and IgM (−21%)
Improvement during placebo	Relative increase from initial to final value[b]	Serum concentrations of albumin (+5 5%) and transferrin (+12%) ESR (−19%)
Deterioration during cyclophosphamide	Relative increase from initial to final value[b]	None
Deterioration during placebo	Relative increase from initial to *final value*[b]	24 hour excretion of transferrin (+38%) serum concentration of creatinine (+7 2%)

Statistical tests [a] Friedman [b] Wilcoxon signed rank
[c] Figures within parentheses indicate the average increases in mean values during period

Variables dependent on the treatment

The effects of cyclophosphamide were analysed in two ways a) by subtracting the values of the variables during the placebo period from the chronologically corresponding values of the cyclophosphamide period for each patient thus forming time series of differences (Fig 1 and Table IV) b) by calculating the relative alteration of the variable in question i e the difference between the last and the first value divided by the average in e[…] of the two treatment periods The first method [of] analysis (*a*) revealed a characteristic pattern [of] improvement during treatment with cycloph[os]phamide in some of the variables including 24 h[our] excretion of protein and albumin albumin cle[ar]ance sieving coefficient for albumin transfer[rin] concentration in urine serum creatinine and IgM [in] serum (*Table IV*) *The alteration consisted o[f]* relative improvement during the first 4 weeks [of] treatment followed by a steady state (Fig 1) [As] evaluated by the second method (*b*) an improv[e]ment was observed during cyclophosphamide tre[at]ment in the following variables 24 hour excretio[n] of protein albumin transferrin and IgA in ur[ine] serum concentrations of albumin transferrin I[gM] and haptoglobin (Table IV) A relative improv[e]ment was also observed during the placebo per[iod] in ESR serum albumin and serum transferrin [No] variables deteriorated during cyclophosphamid[e] whereas serum creatinine and transferrin in ur[ine] increased during the placebo period (Table IV)

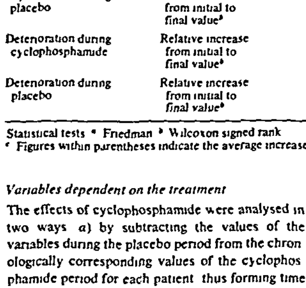


Fig 1 Median value and 75% confidence limits for differences between proteinuria during cyclophosphamide treatment and the chronologically corresponding placebo period

Variables of prognostic significance

The patients were divided into subgroups accord[ing] to the initial subclass or value of the variable[s] and the importance of these variables for t[he] effect of cyclophosphamide on the depende[nt] variables was analysed by a Friedman test

A decrease in serum creatinine was observed i[n]

the presence of normal C4 complement at the start of the treatment initial proteinuria above 2 g/24 h and in the absence of ANA in serum

The effect of cyclophosphamide on proteinuria was demonstrated in a number of subgroups (age above 45 years clinically glomerulonephritis histologically proliferative and membranoproliferative glomerulopathy initially normal BP serum creatinine creatinine clearance C3 and C4 complement absence of ANA and no previous prednisone or azathioprine treatment)

An analysis of the prognostic significance of the variables was also made by correlating the initial values of the variables to the relative changes in a number of the most significant dependent variables Thus a positive correlation between C4 complement and serum albumin was revealed There was also a positive correlation between total haemolytic complement and serum creatinine Finally we found a negative correlation between the C4 complement in serum and urinary albumin

The correlation between relative changes in acute phase reactants or complement fractions and a number of variables dependent upon treatment was analysed The most significant changes were negative correlations between ESR and serum albumin and serum transferrin and positive correlations between ESR and urinary transferrin and urinary IgA

Changes in renal histology

Renal biopsy was repeated in 34 patients during the study In 12 patients the renal histology was assessed before and after the placebo period alone In 22 patients the histology was examined before and after cyclophosphamide treatment (in some cases also including the placebo period)

The type of glomerular lesion (Table II) did not change during the observation period No differences could be demonstrated between the microscopic changes during the cyclophosphamide and the placebo period and we observed no correlation between improvement in the renal state (Table III) and improvement in the microscopic glomerular alterations

Side effects

Seventeen patients developed reversible alopecia Clinical infections were registered in 6 patients 3 of whom had septicaemia One patient died from septicaemia during the study In 5 patients the number of leucocytes in the peripheral blood fell below 2000/μl Information about the risk of gonadal dysfunction during cyclophosphamide did not appear until collection of the present patient material was being concluded which is why this side-effect was not taken into consideration in the planning of the study

DISCUSSION

The results from our investigation demonstrate that 4 months of treatment with cyclophosphamide may yield an improvement in the renal state of the renal glomerular diseases under study The classification of the patients according to normalized improved unchanged or deteriorated renal state represents an attempt to estimate the clinical relevance of the alterations in proteinuria and creatinine clearance even though the basis for this classification is entirely arbitrary The cross-over technique may involve an overlap effect of cyclophosphamide in the placebo period when cyclophosphamide was given first Apparently such an overlap was not pronounced in this study as the shift towards a normalized renal state after cyclophosphamide was independent of whether or not cyclophosphamide was given first (Table III)

The serum creatinine decreased significantly although within the range of normal values in contrast to the marked reduction in proteinuria The decrease in proteinuria as well as in serum creatinine took place mainly during the first 4 weeks of treatment in conformity with the decrease in proteinuria in patients with idiopathic nephrotic syndrome treated with cyclophosphamide (13) On the other hand the effect may be explained by the higher doses of cyclophosphamide during the first 6 weeks of treatment Alterations similar to those observed in proteinuria were registered in urinary albumin transferrin and IgA At the same time there was an increase in the serum concentrations of albumin and transferrin These changes are therefore consistent with a tightning of the glomerular capillaries to proteins of different molecular weight The decrease in the sieving coefficient of albumin demonstrates that the reduction in albumin clearance was not due to a decrease in creatinine clearance The changes in the protein fractions in serum and urine are similar to those observed in our previous semicontrolled study (2) on the effect of azathioprine in renal glomerular diseases

Table IV *Statistically significant alterations in variables during the observation period*

Type of alteration	Observations	Variables showing the alteration[c]
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Statistical tests [a] Friedman [b] Wilcoxon signed rank
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The effects of cyclophosphamide were analysed in two ways *a*) by subtracting the values of the variables during the placebo period from the chronologically corresponding values of the cyclophosphamide period for each patient thus forming time series of differences (Fig 1 and Table IV) *b*) by calculating the relative alteration of the variable in question i e the difference between the last and the first value divided by the average in each of the two treatment periods The first method of analysis (*a*) revealed a characteristic pattern of improvement during treatment with cyclophosphamide in some of the variables including 24 hour excretion of protein and albumin albumin clearance sieving coefficient for albumin transferrin concentration in urine serum creatinine and IgM in serum (Table IV) The alteration consisted of a relative improvement during the first 4 weeks of treatment followed by a steady state (Fig 1) As evaluated by the second method (*b*) an improvement was observed during cyclophosphamide treatment in the following variables 24 hour excretions of protein albumin transferrin and IgA in urine serum concentrations of albumin transferrin IgM and haptoglobin (Table IV) A relative improvement was also observed during the placebo period in ESR serum albumin and serum transferrin No variables deteriorated during cyclophosphamide whereas serum creatinine and transferrin in urine increased during the placebo period (Table IV)

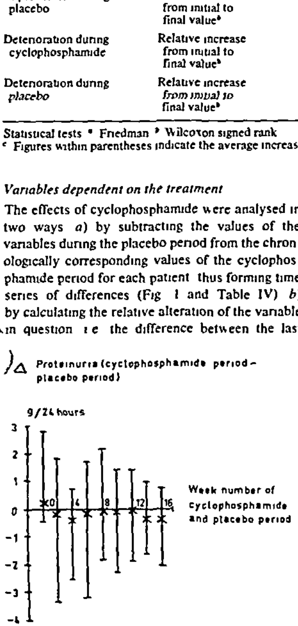


Fig 1 Median value and 75% confidence limits for differences between proteinuria during cyclophosphamide treatment and the chronologically corresponding placebo period

Variables of prognostic significance

The patients were divided into subgroups according to the initial subclass or value of the variables, and the importance of these variables for the *effect of cyclophosphamide on the dependent* variables was analysed by a Friedman test

A decrease in serum creatinine was observed in

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Effects of Chlorthalidone on Serum and Total Body Potassium in Hypertensive Patients

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ABSTRACT Total body potassium has been estimated in 26 hypertensive patients who were hypokalaemic as a result of long term chlorthalidone treatment (mean 20 5 months), while they were on chlorthalidone and 4 weeks after this had been discontinued The mean difference amounted to only 95 mEq (not significant) In 6 additional patients not previously treated with chlorthalidone, serial total body potassium estimations revealed a mean potassium deficiency of 245 mEq after 33 days and of 106 mEq after 100 days These results suggest that the mechanism causing the initial potassium loss is partly reversed or compensated later on In patients with uncomplicated hypertension, no significant potassium deficiency was detected during long term treatment Eighteen of our patients received 39 mEq potassium chloride supplements daily for 4 weeks this caused a mean rise in serum potassium from 3 23 mEq/l to 3 38 mEq/l (not significant) Total body potassium did not change at all We conclude that potassium chloride supplements are not an effective treatment of hypokalaemia in this condition Correction of the extracellular pH by ammonium chloride in 6 patients on chlorthalidone, who demonstrated a slight metabolic alkalosis gave rise to a mean increase in plasma potassium from 2 78 mEq/l to 2 96 mEq/l (not significant) The hypokalaemia in hypertensive patients on long term chlorthalidone treatment cannot be explained by either a potassium deficiency or the change in extracellular pH

A decrease in the serum potassium level is a frequent side-effect during therapy with diuretic drugs Of the hypertensive patients treated with benzothiadiazides (thiazides) or chlorthalidone 24–40% show hypokalaemia (4 10 19 21 23) This phenomenon might be caused by a decrease in total body potassium or by a change in the ratio of extracellular to intracellular potassium concentrations In short term studies an increased urinary potassium excretion has been observed by many authors (6 15 20 24) This of course does not imply that long term treatment will lead to an important potassium deficiency as the mechanism causing kaliuresis might be reversed or compensated

Measurements of exchangeable potassium (3 6 10 12 13 21 26) or total body potassium (1 8 11) during long term treatment with thiazides have been reported These studies show a potassium deficiency ranging from 0 to almost 500 mEq The majority of authors however could not demonstrate a potassium loss of any importance

Only a few papers have been published on potassium deficiency during long term treatment with chlorthalidone in hypertensive patients Healy et al (12) who measured exchangeable potassium reported a mean deficiency of 317 mEq after treatment with 100 mg chlorthalidone on alternate days for 8–15 weeks Remenchik and Johnston (17) found a deficiency of about one fourth the exchangeable potassium during 10–24 months of treatment with chlorthalidone However their patients received an unusually high dose (200 mg daily) furthermore the same patients were not used as controls

Studies of the effectiveness of potassium chloride supplements on serum and total body potassium during diuretic treatment are scarce Results vary from a good effect on serum potassium using a dose of 8 mEq daily (21) to no effect on either serum

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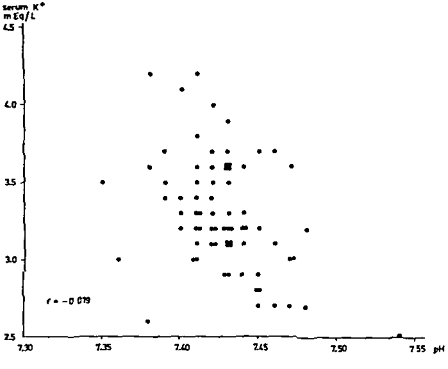


Fig 1 Relationship between serum potassium and blood pH in hypertensive patients on chlorthalidone 100 mg on alternate days

Blood pH and standard bicarbonate were estimated by the method of Siggaard Andersen et al (18)

Total body potassium was estimated by measuring the gamma-ray emission from the naturally occurring radioisotope of potassium ^{40}K using a total body monitor Details of the Leiden total body monitor are described elsewhere (2, 7) ^{40}K countings were taken by three or four sodium iodide crystal detectors with the subject in a prone and in a supine position for 25 min each Depending on the count rate the relative S D of the total body potassium ...uon ranged from 2 9 to 7 6% (22) In four normal ...bjects a total of 15–19 ^{40}K measurements was made in ...course of 8–17 months The S D calculated from this ...ranged from 4 4 to 7 3% which is in agreement with the values based on counting statistics The rather large S D makes it difficult to draw conclusions from measurements in individual patients

The statistical significance of our measurements was calculated by analysis of variance

Table III *Serum potassium and ^{40}K in 6 hypertensive patients in group A at the end of periods a and d*

Periods *a* and *d* as in Table II

Pat no	Serum K (mEq/l) *a*	Serum K (mEq/l) *d*	^{40}K (mEq) *a*	^{40}K (mEq) *d*
19	4 0	3 9	2 687	2 350
20	3 6	4 2	3 229	3 173
21	3 1	3 5	2 493	2 473
22	3 3	3 9	3 874	4 127
23	2 5	3 8	2 511	2 498
24	3 1	3 9	3 242	3 725
25	3 6	3 7	2 248	2 099
26	3 8	3 8	2 721	2 677
Mean Tables II+III	3 27	4 00	2 839	2 934

RESULTS

Body weight and electrolytes

Results of four examinations in 18 patients are presented in Table II results of two examinations in 8 patients in Table III As the results of the first (*a*) examination in Tables II and III and those of the fourth (*d*) examination in Table II and the second (*d*) in Table III are essentially the same, the combined means for all patients in group A have been listed at the bottom of Table III

After discontinuation of chlorthalidone the mean body weight increased from 71 4 to 72 2 kg A mean increase in serum potassium from 3 27 to 4 00 mEq/l was observed This difference is significant ($p<0\ 001$) Our patients showed a slight hypochloraemic metabolic alkalosis during chlorthalidone therapy After discontinuation of chlorthalidone blood pH decreased from 7 44 to 7 40 ($p<0\ 01$) serum chloride increased from 100 1 to 105 2 mEq/l ($p<0\ 01$) and standard bicarbonate decreased from 26 9 to 24 47 mEq/l ($p<0\ 01$)

During diuretic treatment there was no significant

Table IV *Second series of serum potassium and ⁴⁰K measurements in four patients*

Periods *a* and *d* as in Table II Observations in patient 4 were repeated twice

Pat no	Serum K⁺ (mEq/l) *a*	Serum K⁺ (mEq/l) *d*	⁴⁰K (mEq) *a*	⁴⁰K (mEq) *d*
4	3 5	4 5	3 728	4 166
	3 2	4 3	3 766	4 207
7	3 5	4 2	2 535	2 670
14	3 6	4 2	2 759	2 988
24	3 2	3 7	3 649	3 333

correlation between serum potassium and blood pH (Fig 1)

Total body potassium

The results of total body potassium measurements in group A are summarized in Tables II and III During chlorthalidone treatment mean total body potassium was 2 839 mEq After discontinuation the mean was 2 934 mEq The difference is not significant (0 05 $<p<$ 0 10) There was no significant correlation between changes in serum potassium and changes in ⁴⁰K

In 5 patients (nos 2 4 7 14 24) the mean increase in total body potassium amounted to more than twice the S D based on counting statistics This could mean that these subjects did in fact have an important potassium deficiency Therefore measurements were repeated in four of them (nos 4 7 14 24) first after they had been on chlorthalidone again for an average of 5 months then 4 weeks after the drug had been discontinued In the fifth patient (no 2) the measurements were not repeated because the serum potassium had been very low (2 5 mEq/l) immediately after the first series and triamterene was added to her therapy Results are presented in Table IV

Only patient 4 again showed a difference of more than twice the S D Measurements for this patient were taken a third time (Table IV) and still showed a potassium deficiency of similar magnitude We conclude that in this patient chlorthalidone gave rise to an important potassium deficiency Because the clinical condition was not compatible with a potassium deficiency of 877 mEq and as a total body potassium of 3 429 mEq measured the first time, did not recur his potassium loss was probably about 450 mEq and not 877 mEq This patient could not be differentiated from the others by any criteria except his higher serum creatinine Aortic arteriography revealed no renal artery stenosis This patient had none of the complaints usually ascribed to potassium deficiency and ECGs taken on several occasions were normal

Ptassium loss and duration of diuretic therapy

Many authors have reported an increased urinary potassium loss during short term treatment with thiazides or chlorthalidone (15 16 20 24) This would lead to an important potassium loss unless the initial potassium deficit corrects itself during therapy

We studied this problem in 6 patients by means of serial measurements of total body potassium from the beginning of chlorthalidone treatment (group B) Results are presented in Table V and Fig 2 This experiment demonstrated that the lowest total body potassium counts occurred after a mean of 33 days (range 29–43) They were followed by higher counts These results indeed suggest an initial

Table V *Serial measurements of serum potassium and ⁴⁰K during chlorthalidone therapy in the 6 patients in group B*

a=before treatment *b*=after 12–15 days *c*=after 29–43 days *d*=after 75–78 days *e*=after 85–140 days

Pat no	Serum K⁺ (mEq/l) *a*	*b*	*c*	*d*	*e*	⁴⁰K (mEq) *a*	*b*	*c*	*d*	*e*
27	4 0	2 7	2 9	2 8	2 7	3 421	3 296	3 191	3 288	3 165
28	4 2	3 6	3 9	3 6	3 7	3 131	2 797	2 820	2 733	2 920
29	3 7	3 4	3 3	3 5	3 7	4 017	3 907	4 147	3 991	3 995
30	4 1	3 2	3 4	3 4	3 1	3 263	2 974	3 281	3 275	3 355
31	3 8	2 8	2 6		3 1	2 450	2 618	2 133	2 225	2 531
32	4 3	4 2	3 4		4 2	3 549	3 206	2 787	3 178	3 227
Mean	4 02	3 32	3 25	3 32	3 42	3 305	3 133	3 060	3 115	3 199

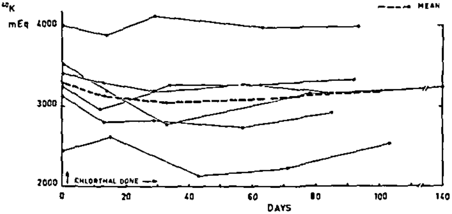


Fig 2 Serial ^{40}K measurements in 6 hypertensive patients on chlorthalidone 100 mg on alternate days Chlorthalidone therapy was initiated in each patient after the first measurement

potassium deficiency that subsequently corrects itself The results in Table V might indicate that the lowest serum potassium level likewise occurred after 4 weeks and then increased Subsequent measurements in these patients after the end of this study did not confirm this suggestion (mean serum potassium 3 18 mEq/l)

Clinical signs of hypokalaemia

Our patients had been using chlorthalidone and were hypokalaemic for a mean of 20 5 months (range 5–52) None of them complained of muscular weakness Some complained of fatigue but it could be attributed to the use of methyldopa

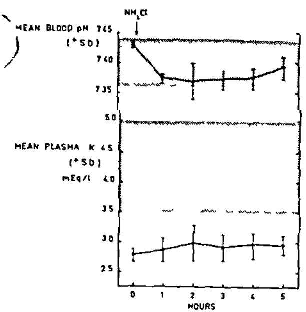


Fig 3 Mean blood pH and plasma potassium in 6 hypertensive patients on chlorthalidone 100 mg on alternate days After the first measurements 0 1 g ammonium chloride/kg b wt was given The shaded areas denote the normal ranges

Myocardial insufficiency was never observed in our patients ECGs were made during and after treatment with chlorthalidone Since hypokalaemia may be accompanied by extrasystoles without ECG signs of hypokalaemia an attempt was made to provoke extrasystoles by having the patient stand However, extrasystoles and other cardiac arrhythmias were never found in our patients Using the criteria of Weaver and Burchell (25) who scored changes of T and U waves and ST depressions only one ECG was characteristic of hypokalaemia. This was made on the day of the second ^{40}K measurement in patient 27 serum potassium was 2.7 mEq/l It is interesting that after subsequent total body potassium estimations the ECGs of this patient were normal although serum potassium remained at the same level Using the same criteria another ECG of patient 2 was suggestive of hypokalaemia serum potassium was 2 7 mEq/l As mentioned above, she was subsequently treated with triamterene

Potassium supplements

The effect of potassium chloride supplements was studied in 18 patients on chlorthalidone The dose was 39 mEq (3 g) daily in three doses for 4 weeks It was given as a solution in order to prevent difficulties in the resorption of potassium chloride The solution was tolerated well Five patients were checked to see if they had taken the right amount of potassium chloride solution by measuring the amount left in the bottle after four weeks During that period these five patients had taken a mean surplus of 67 mEq potassium chloride In our patients potassium chloride supplements caused a mean increase in serum potassium from 3 23 to 3 38 mEq/l (Table II) The difference is not significant No effect on total body potassium was observed

pH correction

Results of the pH correction in six of our patients (nos 3 13 15 18 21 27) are summarized in Fig 3 An ammonium chloride solution was not tolerated well by 2 patients (nos 18 21) who vomited some of it Nevertheless in all six patients the pH fell below a level of 7 40 the mean decrease was 7 43–7 37 Plasma potassium increased only from 2 78 to 2 96 mEq/l

DISCUSSION

Magnitude of potassium loss during diuretic treatment

There is no doubt that in a short term experiment when a thiazide diuretic or chlorthalidone is given to a hypertensive patient an increased urinary potassium excretion will result (14 16 20 24) In the balance studies by Maronde et al (15) who used hydrochlorothiazide the first 10 days resulted in a potassium loss of about 250 mEq Subsequently the potassium balance became less negative This could mean that in time a balance will be reached on a lower total body potassium level However it is also possible that the mechanism causing potassium loss at the beginning is (partly) reversed or compensated at a later stage

To our knowledge there are so far ten studies of hypertensive patients in which body potassium determined as ^{42}K or ^{40}K was measured during long term treatment with thiazides or chlorthalidone (1 3 6 8 *10 11 12 13 21 26) Most of the authors* agree that only a small potassium deficiency less than 200 mEq can be demonstrated But the results differ widely Talso and Carballo (21) and Christensen and Galshow (6) could not demonstrate any potassium deficiency Only Healy et al (12) and Bartorelli et al (3) found an important potassium deficiency (317 and 487 mEq respectively) We have no explanation for this Perhaps the patients of Bartorelli et al suffered from a more severe hypertension associated with secondary hyperaldosteronism It seems unlikely that the difference between pharmacological agents is important in this respect because both Talso and Carballo and Bartorelli et al used hydrochlorothiazide in the same dose It could be that salt intake plays an important role in the origin of potassium deficiency Unfortunately no exact data in this respect are mentioned by the authors In the papers on long term diuretic treatment there is no relation between time on the one hand and potassium loss on the other Healy et al (12) and Bartorelli et al (3) observed an *increase* in potassium deficiency when the diuretic drug was given for a longer period Measurements by these authors were taken between the 2nd and 15th weeks of treatment Gifford et al (10) found a *decrease* in potassium deficiency when chlorothiazide was given for 2–36 weeks If an unusually high dose of a diuretic is given a marked potassium loss may result This was shown by Remenchik and Johnston (17) Their hypertensive patients on chlorthalidone therapy 200 mg daily lost a quarter of the exchangeable potassium

We selected hypertensive patients who were hypokalaemic as a result of chlorthalidone treatment We felt that these patients might show greater potassium loss than patients who were not hypokalaemic Moreover the hypokalaemia proved that these patients were taking their tablets Our results agree with those of the majority of other workers Chlorthalidone treatment in these patients caused no significant potassium deficiency The absence of signs or symptoms of potassium deficiency is in accordance with these findings In one patient a more pronounced potassium deficiency was demonstrated repeatedly This patient differed in no respect from the others except that his glomerular filtration rate was lower We consider that secondary hyperaldosteronism is the most likely explanation Salt restriction during diuretic treatment might influence the potassium loss in two different ways *Firstly it might stimulate aldosterone secre*tion and therefore enhance potassium excretion Secondly during salt restriction a decrease in distal tubular sodium concentration might decrease potassium excretion Unfortunately we did not obtain exact data for estimating the connection between urinary sodium excretion and potassium loss

Serial measurements in our patients suggest a greater potassium loss after a mean of 33 days than after 100 days The results of this study indicate that in hypertensive patients there is a potassium loss during the early stages of chlorthalidone therapy The mechanism responsible is partly reversed or compensated later on

The study by Healy et al (12) might suggest that chlorthalidone gives rise to a more pronounced potassium loss than thiazide diuretics but our results are not compatible with this supposition

Effectiveness of potassium chloride supplements

Results of studies regarding the effectiveness of potassium chloride supplements during diuretic treatment in hypertensive patients vary widely (1 12 21) Talso and Carballo (21) observed an effect on serum potassium of only 8 mEq potassium chloride while Anderson et al (1) could not find any effect on serum and total body potassium of a supplement of 31 mEq daily

In our study 4 weeks of supplementation in the form of an oral dose of 39 mEq potassium chloride daily produced neither a significant rise in serum potassium nor a change in total body potassium Therefore we conclude that potassium chloride supplements in this quantity are not effective in hypertensive patients during diuretic therapy

Possible causes of hypokalaemia during diuretic treatment

We found no significant potassium deficiency in our patients during treatment with chlorthalidone Therefore a change in the ratio of extracellular to intracellular potassium must be at least partly responsible for the decrease in the serum potassium concentration

Talso and Carballo (21) who could not demonstrate any potassium loss suggested that the hypo- was due to the slight alkalosis occurring in condition They observed a normal serum potassium after 4 weeks of ammonium chloride supplements However since chloride could have caused a decrease in the renal potassium excretion *and since Talso and Carballo did not use the same* patients as controls we suggest that their results with long term ammonium chloride administration do not prove the causative relationship between increase in extracellular pH and decrease in serum potassium We found no correlation between serum potassium and blood pH in our patients on diuretic treatment This is in disagreement with the hypothesis of Talso and Carballo

Burnell et al (5) demonstrated that in respiratory alkalosis and in metabolic acidosis a change in the extracellular pH of 0 1 U gives rise to a change in serum potassium of 0 63 mEq/l The change in blood pH (0 035) occurring in our patients is probably too small to account for the decrease in serum potassium (0 73 mEq/l) However to investigate this assumption we studied the effect of correcting the extracellular pH in our hypertensive patients chlorthalidone therapy in a short term experime To limit a possible change in renal reabsorption potassium as a consequence of the chloride su plements we gave ammonium chloride only onc In fact we produced an overcorrection of pl Nevertheless only a slight increase in serum pota sium was observed

We conclude that a change in extracellular p cannot be the only cause of the hypokalaemia hypertensive patients on chlorthalidone treatme In our opinion no satisfactory explanation for th hypokalaemia has yet been found

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Day and Night Work Changes in Cholesterol, Uric Acid, Glucose and Potassium in Serum and in Circadian Patterns of Urinary Catecholamine Excretion

A Longitudinal Cross over Study of Railway Workers

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ABSTRACT Two groups of railway workers ($n=16$ and $n=17$) have been followed on their place of work *during a period of shifts between day and night work* Catecholamine excretion in the urine and blood levels of lipids, glucose, uric acid, potassium and calcium were followed during the different phases of shift work Dramatic fluctuations were noted in the diurnal pattern of catecholamine excretion *during* and *after* night work Significant elevations in the serum levels of cholesterol, glucose, uric acid and potassium were observed during the first week after a night shift, and these changes could not be explained on the basis of shifts in the diurnal pattern or changes in *dietary or other habits*

A recent nation wide survey of the present extent of odd working hours in Sweden has revealed that slightly more than 20% of the working population is employed on some kind of schedule outside regular working hours Half of this group follows regularly or irregularly rotating schedules while the other half can be classified as permanent morning evening or night workers (15) Both halves are often referred to as shift workers

Today there exists a widespread feeling that odd working hours especially across the 24-hour continuum reduce physical psychological and social well being because they interfere with the inherent circadian rhythm of the individual Particularly gastrointestinal nervous sleep and social disturbances have been claimed to be consequences of changing habits of work (1 2 10) However regarding the first two disturbances and also general health and sickness absence the research results are conflicting The main reason for this is the widely acknowledged selection process among the shift workers—those who develop problems largely change over to day work Longitudinal studies of this problem are very rare Other reasons for the lack of conclusiveness are the frequent reliance only on self reported data and the almost exclusive use of non-experimental designs There is furthermore a great lack of information about physiological indices of pathogenic processes involved in the adaptation to shift work

We had the opportunity to obtain physiological data in an experimental design arisen through a group of day workers who were to receive a new temporary work schedule where night work would be scheduled for a period of three weeks This situation made it possible to follow rather intensively what occurs in a group of day workers temporarily exposed to night work Of particular interest was the effect of shift work on parameters connected with psychological and physiological activation and possible costs of such activation To this end a number of psychological and physiological parameters were measured of which the present paper will report on those that may be of some interest to internal medicine namely serum metabolites of cholesterol and total lipids uric acid glucose calcium and potassium Responses to shift work of circadian parameters of urinary catecholamine excretion will also be given Catecholamine excretion has for a long time been used as an indi

Table I *Relative change in serum metabolites for groups A and B in addition to t value for the difference between group means*

	Group A		t value for difference	Group B	
	Mean	S E		Mean	S E
Potassium	0 95	0 02	3 01**	1 03	0 02
Uric acid	0 87	0 04	2 87**	1 02	0 04
Glucose	0 98	0 04	5 08***	1 10	0 02
Cholesterol	0 96	0 03	1 73*	1 02	0 02
Total lipids	0 96	0 04	0 97^NS	1 03	0 06
Calcium	1 01	0 01	1 51^NS	1 03	0 01

* $p<0.05$ ** $p<0.01$ *** $p<0.001$ NS $p>0.05$

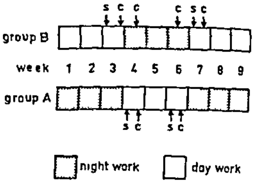


Fig 1 Shift work schedule for the two groups *S*=serum samplings *C*=circadian measures

cator of psychological and physiological activation and has also been suggested to be part of a "wear and tear" pathogenic mechanism (8)

MATERIAL AND METHODS

The participants consisted of two groups of railway workers group A ($n=18$) and group B ($n=18$) comparable as to mean age (42 and 36 years respectively non significant difference) and previous health status anamnestically determined Two subjects in group A and one in group B could not for health reasons participate in the shift work schedule as planned Thus the numbers of participating subjects were 16 and 17 respectively

The work consisted of replacing rail in the daytime mainly of exchanging sleepers and preparing the replacement which was generally carried out at night and was physically rather sternous However work during the night shift usually comprised fewer hours of actual labour than during the day since the work had to be interrupted ... lerable periods when scheduled trains were due The two groups replaced one another in the shift work schedule (Fig 1) Group A had just completed a night shift (22 30–07 30) of three weeks when the study started and then worked in a day shift (7 30–16 30) for three weeks Group B had the opposite schedule

Urine was collected for group B in the fourth 24-hour period of the week just before night work of the first week of night work of the third week of night work and of the first week of return to day work For group A collections were made on the fourth 24 hour period of the first and third weeks of day work Urine samples were obtained at 2–3 hours intervals during the period when the subjects were awake and after termination of the normal period of sleep Hydrochloric acid was added until pH 3 Urine specimens were frozen and the analysis of catecholamine content was made in several series in which all specimens for one individual were analysed on one occasion

The fluorimetric method by Andersson et al (3) was used for analysis This is a semiautomated version of the original method of von Euler and Lishajko (4) The sensitivity and reliability of the new method appear to be very high For comparisons between results obtained by the two methods adrenaline values obtained by the latter method should be multiplied by a coefficient of 1 30 For

Table II *Three way analysis of variance for effects of change between weeks and time of day*
For group B day week "before" is compared to day week "after" and first night week is compared to last night week
For group A comparisons are made between the first and last weeks of day work after the night period

	Time of day		Weeks		Interaction	
	F ratio	d f	*F* ratio	d f	*F* ratio	d f
Group B						
Adrenaline excretion						
Day weeks	9 4***	6/90	0 7	1/15	0 4	6/90
Night weeks	8 6***	7/105	12 5***	1/15	4 7***	7/105
Noradrenaline excretion						
Day weeks	18 1***	6/90	18 9***	1/15	1 0	9/90
Night weeks	6 9***	7/105	2 6	1/15	1 6	7/105
Group A						
Adrenaline excretion day weeks	21 1***	6/96	9 1***	1/16	4 7***	6/96
Noradrenaline excretion day weeks	19 8***	6/96	3 6	1/16	1 4	6/96

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

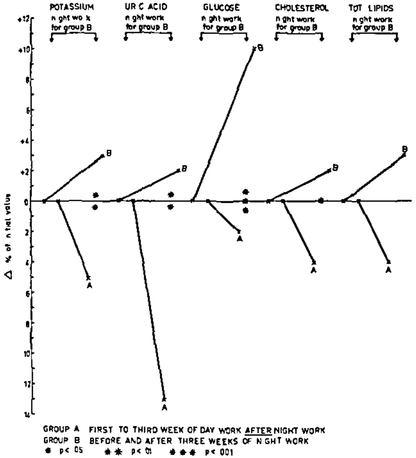


Fig 2 Relative changes in serum metabolites for the two groups

noradrenaline no correction is necessary All subjects were instructed not to consume any alcoholic or coffein containing beverages or drugs and to distribute their tobacco consumption evenly through the day

Blood specimens were obtained from group A at the beginning of the first and the third weeks of day work after the night work (Fig 1) From group B samples were obtained at the beginning of the weeks of day work before and after the three weeks of night work Each subject was kept on a constant schedule for blood drawing throughout the study (the same weekday and hour of day at 6 30–7 30) after at least eight hours of fasting and before any manual work had been performed

Serum cholesterol was analysed according to Zurkowski (16) Serum potassium and calcium were analysed in the flame photometer Total lipids in serum were analysed according to Kunkel et al (6) Serum uric acid was enzymatically analysed according to Morgenstern et al (11) and serum glucose according to Raabo and Torkildsen (13)

With respect to statistical treatment of the serum data second measurement/first measurement ratio was computed for each individual Thus values >1 indicate a relative *increase* and <1 a decrease The means for this relative change were then computed for each group and the difference between the groups was tested for significance (*t* test) (Table I)

To test for effects of time of day and of weeks of measurement for the 24-hour data a three-way analysis of variance according to McNemar (9) was carried out (Table II) This analysis of variance gives all points during the 24 hours equal weight despite the fact that e g sleep values represent a considerably longer time span than any separate period during the day Considering the usually encountered low values of sleep excretion this results in a somewhat lowered probability of receiving a significant *F* quotient for time of day

RESULTS

The group means of the relative changes in each variable together with statistical significance levels for the differences between the groups (one tailed *t* test) are shown in Table I and Fig 2 On the whole group A recovering from night work showed *decreased* levels from the first to the second measurement (ratio <1) whereas group B be

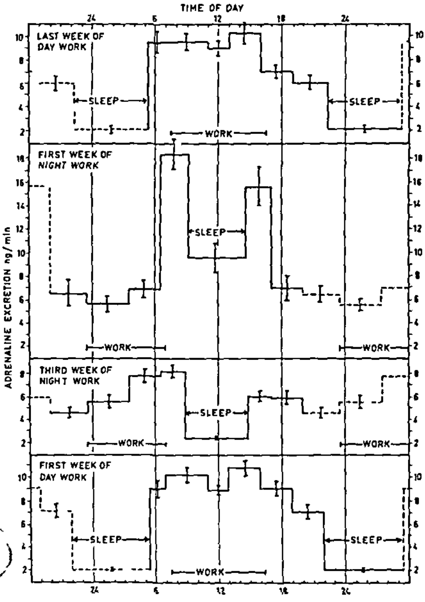


Fig 3 Adrenaline excretion before during and after night work (group B) (mean and S E)

ing exposed to night work showed *increased* levels from before to after night work (ratio >1) These differences in pattern were statistically significant for potassium uric acid glucose and cholesterol but not for total lipids

Means and S E of 24-hour patterns of noradrenaline and adrenaline excretion for groups A and B are shown in Figs 3–6

From Fig 3 it is evident that the night work caused marked effects on the adrenaline patterns for group B The first night week exhibits a very high mean level while the third week of night work shows a flattened pattern However when patterns before and after the period of night work are compared (Table II) no difference is seen in adrenaline excretion while the noradrenaline excretion is significantly increased The 24 hour excretion of both catecholamines showed highly significant circadian patterns for the day weeks

The change in the total 24 hour excretion of adrenaline from the week before night work to the first week of night work was significant (t=3 36 p<0 01, two-tailed) The corresponding t value for the comparison between periods of sleep during the two days and nights of study was 2 95 (p<0 05) and for periods of wakefulness 1 17 (N S) Thus

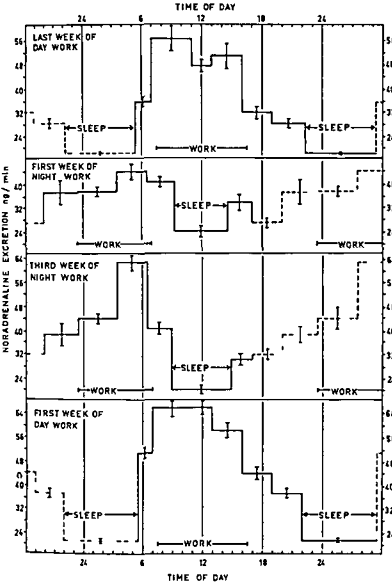


Fig 4 Noradrenaline excretion before during and after night work (group B) (mean and S E)

there was a general increase in the level of adrenaline excretion during the first week of night work The 24-hour excretion of noradrenaline did not show a significant change during these periods

The change in the 24-hour excretion of adrenaline from the last week of night work to the first week of day work was also significant ($t=2$ 30 $p<0$ 05) The corresponding t value for the comparison between periods of sleep during the two days and nights of study was -1 13 (N S) while the comparison between periods of wakefulness for these days and nights yielded a t value of 3 11 ($p<0$ 01) Thus the adrenaline excretion increased strikingly during periods of wakefulness *after* night work but even tended to decrease during periods of sleep after night work The same tendencies were observed for noradrenaline (24-hour excretion $t=1$ 99 N S periods of sleep $t=-0$ 26 N S periods of wakefulness $t=2$ 52 $p<0$ 05)

Fig 4 reveals that also the catecholamine patterns for group A during the first and the third week after night work showed very distinct circadian patterns (Table II) For noradrenaline no significant change between the weeks could be detected For adrenaline however there was a significant increase in overall level from the first to the third

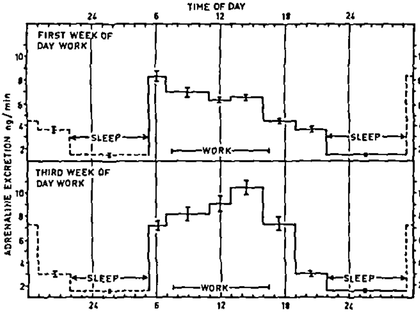


Fig 5 Adrenaline excretion during the first and third week after night work (group A) (mean and S E.)

week of day work Also the interaction term was significant implying that also the shape of the two patterns differed i e 24 hour excretion immediately after night work had a somewhat earlier phase than during the third week of day work

DISCUSSION

Diary data did not indicate any changes over weeks in coffee/tea tobacco or alcohol consumption Neither were any changes noted concerning amount or kind of food or physical activity Thus the results could hardly be due to these factors The physiological variables studied were selected in order to illuminate different biological functions adrenomedullary activity and carbohydrate lipid and nucleic acid metabolism and electrolyte balance (potassium and calcium)

The results indicate a dramatically increased level of catecholamine excretion during the first week of night work as well as a distorted phase pattern during the third night week After the return

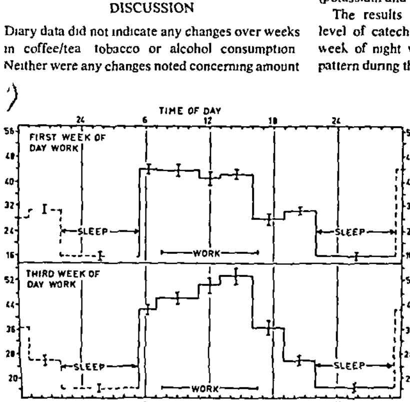


Fig 6 Noradrenaline excretion during the first and third week after night work (group A) (mean and S E)

to day work the level and phase pattern of adrenaline excretion appears to have retrieved most of the before pattern very fast while the level of noradrenaline excretion was significantly higher although the phase pattern was normalized Group A studied over the three weeks of recovery on normal day time did not show any change in noradrenaline pattern Adrenaline excretion however was somewhat depressed immediately after night work The significant *F* ratio for interaction adds the further information that the phase tended to occur later during the third week than during the first From the practical standpoint the study points out the importance of evaluating the subjects sleeping patterns when serum metabolites or catecholamine excretion are analysed

It could be argued that part of the change in blood parameters may have been due to the lagging of changed diurnal patterns which may thus have caused elevated morning levels This however seems highly unlikely to judge from the literature on the circadian rhythms of most of the parameters studied (5 12 13) as the variations found in this study exceeded what may be expected even from an unlikely maximal phase lag of the curves No significant tendency was however found in the present study for *total lipids* Serum triglycerides which form a large proportion of the total serum lipids are known to exhibit large variations between day and night levels Thus in this case shifts in circadian patterns are likely to influence the total lipid pattern (5 7) Furthermore the phase of catecholamines as well as body temperature (not reported here) in the after measure did not differ from the before measure supporting the interpretation that differences were not caused by phase lags It is necessary however to perform repeated sampling of serum metabolites in future studies to make possible a more exact evaluation of the effects of inversion of the rest–activity cycle

Catecholamines are known to give rise to hemoconcentration This could partly explain the changes in serum levels of the metabolites However in the present study such an explanation is unlikely A previously published study of sleep deprivation during three consecutive days and nights did not indicate significant changes in hematocrit (8)

It appears that the overall metabolic situation associated with night shift work could be interpreted as catabolic and energy providing The present study was not designed for studying the long term health consequences of the night shift work Such a study would require larger samples and longer observation periods However it could be argued that long standing elevations of catecholamine excretion as well as changes in the levels of lipids and electrolytes may be parts of a pathogenic mechanism which eventually may affect e g the cardiovascular or other systems

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Clofibrate in Type II Hyperlipoproteinemia

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ABSTRACT As part of a double blind randomized study, the safety and the lipid and uric acid lowering effect of clofibrate have been evaluated in 28 patients with type II hyperlipoproteinemia (HLP) A highly significant reduction of serum cholesterol occurred in type II a and of serum triglyceride and cholesterol in type II b HLP throughout the 60-week observation period ($p<0\ 01$) Of the patients with types II a and II b HLP, 65% had at least a 25% reduction of serum cholesterol Uric acid was significantly reduced only during the first period of treatment ($p<0\ 05$) In the laboratory measurements concerning safety, a persistent, slight reduction was observed in Hb, hematocrit and alkaline phosphatase No significant clinical side-effects were noted Clofibrate is considered effective as a lipid lowering agent in many cases of type II HLP

Although the lipid lowering effect of clofibrate has been repeatedly demonstrated in hyperlipoproteinemias (HLP) it is not infrequently maintained that the serum cholesterol levels in patients with type II HLP (essential cholesterolemia hyper β lipoproteinemia) respond poorly to clofibrate therapy (12 13 14 20) The result of the present double blind randomized study supports the contention that clofibrate is also effective in the majority of cases of type II HLP

MATERIAL AND METHODS

After a thorough discussion of the purpose and procedure of the invesugation patient volunteers ranging in age from 44 to 65 years (mean 55) with type II HLP and persistent elevation of serum cholesterol (>280 mg/100 ml) were selected for this double blind study (clofibrate and an unregistered hypolipoproteinemic drug) Patients with secondary causes of HLP were excluded Twenty of the patients had atherosclerotic complications All patients had been on appropriate dietary therapy prior to the study when β lipoprotein was high a low saturated high polyunsaturated diet low in cholesterol was prescribed patients with high pre β lipoproteins as well were instructed to moderate their sugar intake and lose weight They were told to continue on the same diet and to avoid excessive alcohol intake throughout the study When the code was broken clofibrate had been given to 10 male and 18 female patients of whom 8 had type II a and 20 type II b HLP according to Fredrickson s classification (7 8)

The investigation period was divided into a placebo period of 8 weeks and 5 treatment periods each lasting 12 weeks Blood determinations were made initially every 4 weeks during the placebo period and the first treatment period and once during each of the subsequent 4 treatment periods All patients were given placebo or clofibrate (2 g daily) in the form of two 500 mg capsules twice daily

Blood for lipid analyses was drawn at each visit to the clinic after an overnight fast Cholesterol was determined by the method of Runde (16) plasma triglyceride by the method of Eggstein and Kreutz (6) and the typing of HPL was made by lipoprotein electrophoresis (5) Serum uric acid Hb hematocrit WBC platelets fasting blood sugar serum creatinine serum bilirubin serum alkaline phosphatase and SGPT were determined by routine laboratory procedures

Wilcoxon s rank test and Student s *t* test for paired data were used for statistical analyses

RESULTS

Cholesterol and triglycerides

The lipid lowering effect of clofibrate is shown in Table I The mean cholesterol reduction in both types II a and II b was approximately 20% (variations 0–60%) At least a 25% reduction of serum cholesterol was found in 65% of the patients The mean reduction of plasma triglycerides in type II b was approximately 30% (variations 0–75%) At least a 30% reduction of plasma triglycerides was found in 65% of the patients No significant change

Table I *Effect of clofibrate on serum cholesterol plasma triglycerides and uric acid (mean±S E M)*

	Placebo period (0–8 weeks)	Active treatment periods (weeks) 1st (8–20)	2nd (20–32)	3rd (32–44)	4th (44–56)	5th (56–68)
Type II a HLP						
No of pats	8	8	8	5	–	–
Serum cholesterol (mg/100 ml)	368±19 4	292±19 6	305±26 5	311±31 4	–	–
Reduction (%)	–	20 8± 3 0	17 8± 3 9	15 6± 6 5	–	–
P value	–	<0 001	<0 001	<0 01	–	–
Type II b HLP						
No of pats	20	20	19	11	7	7
Serum cholesterol (mg/100 ml)	403±16 0	315±15 5	319±14 5	296±20 8	287±14 8	285±15 6
Reduction (%)	–	22 1± 2 8	19 6± 3 1	25 5± 4 2	26 0± 5 7	29 5± 4 8
No of pats with ≥15 % reduction	–	14	11	9	6	7
P value	–	<0 001	<0 001	<0 001	<0 01	<0 01
Plasma triglycerides (mg/100 ml)	313±32 6	202±16 2	203±24 2	175±19 7	179±31 1	175±21 9
Reduction (%)	–	31 9± 4 1	32 3± 5 1	33 7± 6 4	36 7± 8 1	39 7± 5 5
P value	–	<0 001	<0 001	<0 001	<0 01	<0 01
No of pats	18	18	17	9	–	–
Plasma uric acid (mg/100 ml)	4 9	4 4	4 9	5 5	–	–
P value	–	<0 05	n s	n s	–	–

n s =non significant

in plasma triglyceride levels which were within the normal range prior to clofibrate treatment was observed in type II a

ric acid

)significant reduction of mean uric acid from 4 9 o 4 2 mg/100 ml was present only in the first clofibrate period ($p<0\ 05$) (Table I)

Safety data

Table II summarizes the safety laboratory measurements showing the significance of the changes between the placebo period and each active period

Hb and hematocrit were slightly reduced in all three treatment periods ($p<0\ 01$) Total WBC was slightly reduced during the first and second active medication period ($p<0\ 01$) Fasting blood sugar

Table II *Effects of clofibrate on safety measurements*

	Placebo period (*n*)	Placebo period (Mean)	Active treatment periods (weeks) 1st *n*	1st Mean	2nd *n*	2nd Mean	3rd *n*	3rd Mean
Hb (g/100 ml)	18	14 9	18	14 3**	17	13 9***	16	14 0 *
Hematocrit (%)	18	45 3	18	43 4***	17	43 6***	16	43 9
WBC (1 000/ml)	18	8 18	18	7 28**	17	6 44***	14	7 16
Platelets (1 000/ml)	18	244 3	18	259 1	17	265 1	14	?33 9
Fasting blood sugar (mg/100 ml)	18	87 5	18	79 4***	17	80 2**	14	89 4
Serum creatinine (mg/100 ml)	18	0 97	18	1 03*	17	0 98	14	0 99
Serum bilirubin (mg/100 ml)	18	0 48	18	0 39 *	17	0 40	14	0 39
Serum alkaline phosphatase (U/l)	18	34 6	18	21 1***	17	15 9***	14	18 9**
SGPT (U/l)	18	7 6	18	8 9	17	8 9	14	11 9

Significant difference from mean placebo values *$p<0\ 05$ **$p<0\ 01$ ***$p<0\ 001$

and serum bilirubin were significantly reduced during the first and second medication period as was serum alkaline phosphatase during all medication periods ($p<0.001$) No significance was found for changes in SGPT

Adverse reactions

A clinical adverse reaction was suspected in only one patient who complained of worsening of her spastic colitis during the first month of clofibrate treatment

DISCUSSION

Clofibrate has been available for more than 10 years and a number of studies have demonstrated its safety and efficiency It is primarily a triglyceride lowering agent and exerts its most profound effects in patients with type IV HLP (2 14) Clinical experience of treatment with clofibrate in patients with type II HLP is limited and there are few well conducted double blind studies on its ability to lower serum cholesterol

Our data show that clofibrate is a potent lipoprotein lowering agent in many cases of type II HLP As demonstrated previously (1 3 11 17) the lipid lowering ability of clofibrate was pronounced in patients with type II b HLP the mean decrease in triglyceride levels in such patients being more than 30% and the decrease in the cholesterol levels approximately 20% Our results also show that a number of subjects with type II a HLP respond and that the cholesterol lowering effect is almost equivalent to that seen in type II b At least a 25% reduction of serum cholesterol was observed in 65% of our patients with types II a and II b HLP In no case did clofibrate treatment significantly increase the serum cholesterol levels suggestive of a rise in β lipoprotein (15) The side effects were limited to minor gastrointestinal complaints in a single case

Among the safety parameters there was a persistent reduction of the slightly elevated Hb and hematocrit pretreatment values during the clofibrate medication The reason for this normalization is not known However we have reported previously (4) that high plasma triglyceride levels may be associated with an increase in the oxygen affinity of whole blood With a significant reduction of plasma triglyceride the release of oxygen from the erythrocytes through the microcirculation may increase leading to a compensatory lowering of the Hb and hematocrit levels Likewise a persistent significant reduction of serum alkaline phosphatase was found an observation which has been reported before (10 18) The mechanism of this reduction is not known The addition of serum from clofibrate treated patients to serum samples from untreated individuals did not result in any significant variation in the expected level of alkaline phosphatase in the mixtures (18) Therefore it seems unlikely that clofibrate interferes with the determination of alkaline phosphatase

The mode of action of clofibrate is still not clearly understood although recent studies have greatly furthered our knowledge (3 9 19) Clofibrate appears to lower plasma cholesterol by increasing the excretion of neutral steroids in the faeces by decreasing cholesterol synthesis and by reducing the tissue pool of cholesterol Whether or not this includes removal of cholesterol from the vascular walls has not been determined

Since both hypercholesterolemia and endogenous hypertriglyceridemia are associated with increased risk of coronary heart disease it seems recommendable to treat at least severe degrees of HLP

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Treatment of Hypercholesterolaemia and Hypertriglyceridaemia with Magnesium

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ABSTRACT A daily intake of 3 g magnesium (as magnesium oxide) for six weeks had no effect on serum cholesterol and serum triglyceride in 17 patients suffering from hypercholesterolaemia and/or hypertriglyceridaemia

It is well known that a high level of serum lipids is one of the most significant risk factors for myocardial infarction (MI) (14) and atherogenesis (13) Animal experiments have shown that a magnesium deficient diet produces an increase in serum cholesterol which could be normalized by adding an appropriate amount of magnesium to the fodder (5) A similar inverse relationship between serum levels of cholesterol and magnesium was observed in Bantus by Bersohn and Oelofse (2) Maize meals the staple diet of the Bantu reduce the serum cholesterol levels and increase the magnesium levels in white healthy males (3)

Vitale et al (15) found a highly significant inverse relationship between the magnesium content in the food and the amount of lipid deposited in the heart and aorta in rats Furthermore they demonstrated that the atherosclerotic lesions were diminished by increasing the magnesium intake In patients suffering from cardiovascular disease it is still not clear whether magnesium deficiency (as expressed by low serum magnesium) is correlated to lipaemia (4 7 9)

The aim of this study was to examine whether magnesium supplements decrease the serum levels of triglyceride and/or cholesterol in patients suffering from hypertriglyceridaemia and/or hypercholesterolaemia

PATIENTS AND METHODS

Included in the study were ten males and seven females with a mean age of 49 years (range 22–69) The criterion for selection was a serum level of cholesterol and/or triglyceride above the normal range ($\bar{x}$+2 S D) for healthy sex and age matched individuals All patients had normal serum levels of creatinine sodium and potassium Four out of the 17 patients had previously had MI but none had cardiac incompensation The patients were checked regularly at the Lipid Clinic in Glostrup Hospital and all had been without antilipaemic treatment for at least three weeks before the study

Serum magnesium and serum calcium were measured by atomic absorption spectrophotometry (Perkin Elmer 403) and corrected to a constant serum protein level (6) Serum cholesterol was measured by the method of Grafnetter et al (8) and serum triglyceride by the method of Laurell (11) All measurements were made in duplicate and the coefficients of variation of duplicate measurements are given in Table II

Blood samples were drawn initially (time t_0) after six weeks (time t_1) and after nine weeks (time t_2) All specimens were drawn after nine hours fasting From t_0 to t_1 the patients were treated with magnesium oxide orally (3 g magnesium daily) in the period t_1–t_2 they were untreated

The therapeutic trial was conducted from June to Sept 1974 Student s t test for paired differences was used to evaluate the effect of treatment and a test for linear correlation was used to evaluate the relations between the biochemical parameters examined

RESULTS

Table I gives the coefficients of correlations between the determined biochemical parameters at time t_0 A significant inverse correlation was found between the serum levels of triglyceride and calcium No significant relationship was found between initial values of serum magnesium and serum triglyceride (r=0 05 p>0 05) or between serum

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Studies on Subclinical Hypothyroidism with Special Reference to the Serum Lipid Pattern

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ABSTRACT Subclinical hypothyroidism is an example of the impact of technology on the concept of a disease It denotes a condition in which laboratory findings, at least including a raised serum thyrotropin (s-TSH), indicate hypothyroidism in the absence of clinical signs or symptoms of this disease One reason for attention to cases of subclinical hypothyroidism is the publication of reports, from the time before introduction of the s-TSH assay, that hypercholesterolaemia precedes other evidence of thyroid failure with attendant risks of ischaemic heart disease and other atherosclerotic manifestations The present investigation, which concerned the lipid pattern in subclinical hypothyroidism offered no support for such a concept of hypercholesterolaemia as a premonitory sign of hypothyroidism Furthermore, no significant differences were found between the serum levels of cholesterol and triglycerides before and after the administration of a thyroxine dose, necessary to suppress the s-TSH into a normal range in cases of subclinical hypothyroidism Nor were there any changes during this therapy in body weight ECG or Hb levels, which represent important parameters often found to be abnormal in overt hypothyroidism From a practical point of view, subclinical hypothyroidism probably can be regarded as a state in which reduction of thyroid activity has been compensated by an increased s TSH secretion to maintain a clinically euthyroid state When no goitre is found the rationale of treatment of this condition remains to be proved

The clinical syndrome of hypothyroidism was first described one century ago by Gull (9) The term myxoedema was introduced somewhat later by Ord (14) This term denotes the particular picture of advanced hypothyroidism with marked swelling of the subcutaneous and submucosal tissues resulting in such well known symptoms as a swollen face and hoarseness of the voice The advanced myxoedematous forms of hypothyroidism are seldom seen nowadays and they hardly represent any substantial diagnostic or therapeutic problems for a physician equipped with elementary clinical education and modern laboratory and pharmaceutical facilities On the contrary by pushing back the point of recognition of suboptimal thyroid hormone production to an earlier phase in the spectrum of hypothyroidism improved laboratory techniques have created a new problem viz the management of patients with laboratory findings indicating hypothyroidism but with absence of clinical signs and symptoms of this disease A great number of such patients are found in for instance posttherapy follow ups of hyperthyroid patients treated with surgical or radioiodine ablation of thyroid tissue Terms such as subclinical hypothyroidism (5) latent hypothyroidism (17) and premyxoedema (6 7) have been coined for this particular form of hypothyroidism which is recognized only by biochemical abnormalities The two latter terms are not quite suitable as they presuppose that overt hypothyroidism eventually develops in these cases which has not been satisfactorily proved In the present paper we therefore prefer to use the term subclinical hypothyroidism and define this condition like Evered et al (5) as the combination of absent clinical signs and symptoms of hypothyroidism with an elevated level of serum thyrotropin (s TSH) which is the most sensitive laboratory test nowadays employed for detecting suboptimal production of calorigenic thyroid hormones

One suggested reason for careful attention to subjects with subclinical hypothyroidism is the possi

Table 1 Serum levels of thyrotropin cholesterol and triglycerides before and after normalization of s TSH among 29 patients with subclinical hypothyroidism

	Before thyroxine			After thyroxine		
	Range	Mean	S D	Range	Mean	S D
TSH (mU/ml)	9–33	19	8	2–8	5	2
Cholesterol (mg/100 ml)	157–316	222	41	132–310	215	40
Triglycerides (mg/100 ml)	65–338	148	65	65–296	144	59

bility of hypercholesterolaemia secondary to this condition (6 7) This may in the long run contribute to atherosclerotic coronary and peripheral vascular disease (2 3 8 12 22) Evidence of hyper cholesterolaemia as a sign of clinical importance preceding other signs of hypothyroidism has been presented by Fowler et al (6 7) However Eve red et al (5) found no hypercholesterolaemia in subclinical hypothyroidism In this study we therefore determined serum cholesterol as well as serum triglycerides before and after thyroxine replacement in subjects with subclinical hypothyroidism The aim was to detect any hyperlipidaemia or any substantial lowering of the serum lipid levels following thyroxine treatment with the dose required to normalize s TSH levels Thyroxine treated subjects with originally overt hypothyroidism were also included in the group of subclinical hypothyroidism if they were devoid of any clinical signs or symptoms of hypothyroidism even though the dosage of thyroxine was insufficient to suppress s-TSH into a normal range

In cases of subclinical hypothyroidism we also searched for changes in the ECG body weight and Hb level which represent important parameters often found to be abnormal in overt hypothyroidism

PATIENTS

The material consisted of 29 patients (5 men and 24 women) mean age 54 Seventeen patients had not been treated previously with thyroid hormone and 12 had a suboptimal thyroxine substitution of 0 05–0 10 mg thyroxine per day The patient group included 18 posttherapy cases (10 cases with radioiodine and 8 with surgical ablation of the thyroid) The surgical procedure had invariably consisted of subtotal resection of a toxic goitre No patient had any goitre at the time of examination Two had an endocrine ophthalmopathy with exophthalmus and swollen eye lids

In order to verify the clinical impression of euthyroidism a diagnostic index described by Billewicz et al (2) was used This index is built up from signs and symptoms of hypothyroidism generally accepted as useful in clinical evaluation

A replacement dose of 0 05 mg thyroxine (Levaxin® Nyegaard) per day was used for untreated patients In all but two cases this small dose was sufficient to normalize the s TSH levels In the two exceptions another 0 05 mg i e totally 0 1 mg thyroxine was found to be necessary to suppress s TSH to a normal level In the 12 patients already substituted with thyroxine elevated s TSH was normalized by adding 0 05 mg to the previous dose of 0 05 or 0 10 mg thyroxine Laboratory examinations were performed before and 6 weeks after alteration of the thyroxine dose The blood samples for analysis were drawn in the morning with the patients fasting

METHODS

S TSH was measured by radioimmunoassay using a commercial kit Phadebas® TSH test (Pharmacia Uppsala Sweden) The upper limit among normal persons has been found to be close to 8 μU/ml Values at about this level were therefore considered to be abnormal

S thyroxine was determined by column chromatography using an automated method for the final reaction (16) The reference range is 57–145 nmol/l

T resin uptake test was performed using Sephadex as absorbent The method is a modification of the method described by Hansen (10) Reference range for men 80–120% uptake for women 75–115% uptake of the resin uptake from a sample of pooled serum

S cholesterol and s triglycerides were determined simultaneously after extraction with isopropanol using Zeolit and Fuller earth for absorbing phosphatides (18 19) Reference range for s cholesterol 150–300 mg/100 ml (4 0–7 8 nmol/l) and for s triglycerides 50–180 mg/100 ml (0 60–2 20 nmol/l) B hemoglobin was determined by the hemoglobin-cyanide method (11) The ECGs were registered by an ordinary 12-channel mingograph

RESULTS

No patient reported any symptomatic change during the adjustment of s TSH to a normal level Particular attention was then paid to body functions which may reflect thyroid function such as bowel

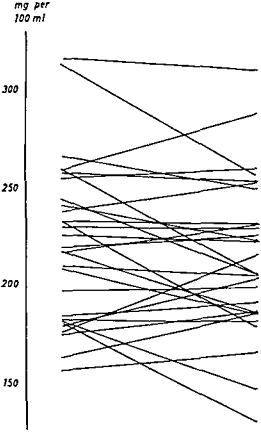


Fig 1 Serum cholesterol levels before and after suppression of serum thyrotropin into the normal range

habits skin dryness cold tolerance or need for sleep The two patients with endocrine ophthalmopathy showed no change in their eye symptoms during the six week period of hormonal substitution

The s TSH levels (Table I) before thyroxine suppression ranged from 9 to 33 mU (mean 19 S D 8) After replacement with thyroxine all patients had by definition a s TSH level not exceeding 8 which was the upper limit of the normal range

The serum cholesterol (Table I) before adjustment of the s TSH levels ranged from 157 to 316 mg/100 ml (mean 222 S D 41) (Fig 1) The range after suppression of s TSH to a normal level was 132–310 (mean 215 S D 40) The small difference in the mean serum cholesterol levels was not statistically significant When defining hypercholesterolaemia in clinical practice 300 mg cholesterol/100 ml is a commonly used cut-off point in the normal distribution of this parameter Only 2 of 29 patients had a pretreatment serum cholesterol above this level

There was no significant difference in serum triglycerides (Table I) before (mean 148 mg/100 ml S D 65) and after (mean 144 mg/100 ml S D 59) adjustment of s TSH levels (Fig 2) In five cases pretreatment serum triglycerides were above 200 mg/100 ml—a point in the normal distribution often chosen to delineate hypertriglyceridaemia

No significant change was found in body weight or Hb levels during normalization of the serum thyrotropin level Nor was there any significant difference in the ECG as to PR duration QRS duration QRS amplitude R–R interval or T wave configuration which parameters are often definitely abnormal in overt hypothyroidism

The mean serum thyroxine level (Table II) before suppression of s TSH to a normal level was 55 (S D 19) nmol/l (9 of 17 patients below the normal range) among untreated patients and 67 (S D 13) nmol/l (2 of 12 patients below the normal range) among incompletely thyroxine substituted patients The corresponding figure after normalization of s TSH was 79 (S D 14) and 95 (S D 27) respectively No patient had serum thyroxine values below the normal range after thyrotropin had been normalized

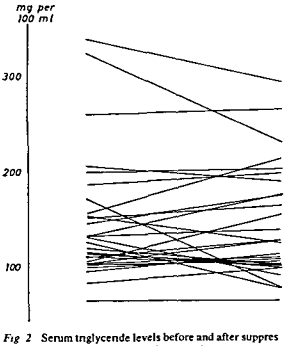


Fig 2 Serum triglyceride levels before and after suppression of serum thyrotropin into the normal range

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The Composition of Food Consumed by Greenland Eskimos

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ABSTRACT Food specimens have been collected, by means of the double-portion technique, from Greenland Eskimo hunters and their wives, in all seven persons, on seven consecutive days Their food was found to contain more protein and less carbohydrates than average Danish food and an almost equal amount of fat Compared with Danish food, the fatty acid pattern of the consumed lipids—essentially of mammalian marine origin—showed a higher content of long chain polyunsaturated fatty acids (especially C20 5) *and lower contents of linoleic and linolenic* acids However, the sum of the polyunsaturated fatty acids was smaller than in Danish food Using Keys' formula, describing the serum cholesterol level as a function of the nutritional fatty acids, the essentially lower serum cholesterol level found in Greenland Eskimos was not explained by our findings It is suggested instead to be a special metabolic effect of the long chain polyunsaturated fatty acids from marine mammals There might be a similar effect on the plasma triglyceride and very low density lipoprotein concentrations, explaining the much lower plasma concentrations of these components in Eskimos than in Western populations Our findings might have an essential bearing on the difference in morbidity from coronary atherosclerotic disease between these populations

In the summer of 1970 the serum lipid pattern was examined in 130 Greenland Eskimos in the Umanak district in the northern part of West Greenland (2) Compared with people living in Denmark the levels of serum cholesterol and β lipoprotein were relatively low The serum triglyceride level was much lower and consequently the pre β lipoprotein concentration in serum was very low

Furthermore the fatty acid patterns of the three main serum lipid classes—cholesterol esters triglycerides and phospholipids—were examined (4) and major differences were found compared with people living in Denmark In summary for all three serum lipid classes the Eskimos living in Greenland displayed lower concentrations of linoleic linolenic and arachidonic acids and higher concentrations of palmitic palmitoleic timnodonic and docosahexaenoic acids

In the same investigations the serum lipid pattern of Eskimos living in Denmark was examined and found to be essentially similar to that of other Danes living in Denmark also the plasma fatty acid pattern was similar As a result it was concluded that genetic factors were of minor importance for the differences which consequently must be attributed to external living circumstances presumably the diet of the Eskimo community in Greenland

Coronary occlusion is uncommon in Greenland Eskimos This fact is in accordance with the low serum lipid levels of this population (2)

It must be considered probable that the serum fatty acid pattern which is so different from that of people from the Western community is a result of the fatty acid content in the Eskimo food If so a different fatty acid composition of the Eskimo diet could be one of the main reasons for the low plasma lipid concentration and hypothetically for the low incidence of coronary occlusions

In order to elucidate any accord between the serum fatty acid pattern and the fatty acid composition of the dietary fats consumed by Eskimos the composition of their diet was investigated The study was carried out during the summer of 1972—the same season in which the serum lipid study was undertaken—and on the same population

Table I *Average amounts of protein fat and carbohydrate (calorie %) in the diet of arctic populations and of the Danish population in 1972*

Authors	Year of publication	Place of study	Protein	Fat	Carbo-hydrate
Krogh & Krogh (13)	1914	Northwest Greenland	44	47	8
Berthelsen (3)	1935–43	Greenland			
		Hunters	19	27	53
		Fishermen	40	10	50
Rodahl (14)	1954	Alaska		37	
Uhl et al (16)	1955	Northwest Greenland	36	25	39
Bang & Kristoffersen (1)	1972	Alaska inland			
		In 1955–57	33	41	26
		In 1965	15	40	45
Feldman et al (6)	1972	Northwest Greenland	30–35	50	15–20
Present investigation	1974	Northwest Greenland	26	37	37
Helms (10)	1972	Denmark	11	42	47

SUBJECTS MATERIAL AND METHODS

Seven persons were included in the study five males and two females aged 32–76 years They were selected so as to give the best impression of eating habits in the small settlement of Igdlorssuit which is situated on an island in the Umanak district at latitude 71° N about 500 km north of the Arctic Circle on the west coast of Greenland All the subjects also participated in the serum lipid study (2) The inhabitants of Igdlorssuit (about 140 persons) all live to a greater or lesser degree on the catch from hunting and fishing predominantly seal whale and fish There is a little shop in the settlement run by the Royal Greenland Trading Company from which some food is available primarily bread biscuits sugar margarine potatoes rice coffee tea milk powder flour and beer

During a period of seven days all meals of the seven subjects were collected and weighed in accordance with similar nutritional studies by Keys and Kimura (12) The subjects were asked not to change their habitual diet and to collect and deliver quantitative duplicates of all their meals They were visited once a day and interviewed concerning the composition of the meals on the previous day After collection the food specimens were weighed homogenized and aliquots were taken out and kept frozen until they could be analyzed in Denmark

The following analyses were carried out water content after freeze drying and weighing of the residue protein content by the Kjeldahl method content of fat after extraction and weighing by the method of Folch et al (7) and content of salts after burning by weighing of the ashes The carbohydrate content was calculated as the difference between dry weight and the sum of proteins fats and salts The caloric values were calculated by means of Atwater s factors (protein and carbohydrate 4 fat 9 kcal/g)

In the fat extracts cholesterol was determined by gas-liquid chromatography on a 3% OV column at 260°C The Danish State Institute of Food assisted us in these determinations The fatty acids were determined—after hydrolysis and methylation—by gas liquid chromatography using a Beckman GCM gas chromatograph equipped with a 2 m glass column of 3 mm i d The supporting medium was chromosorb W AW mesh 80–100 and the stationary phase 10% w/w diethylene glycol succinate Analyses were carried out at an injection temperature of 250°C a column temperature rising from 150 to 200°C during the run and at a column flow of 30 ml nitrogen/min A hydrogen flame ionization detector was used at 250°C The signal was analyzed by a disc integrator and compared with those of assays on mixtures of fatty acids supplied by Nu Check Prep Elysian Minnesota USA Analytical grade petroleum ether was used as solvent and checked for impurities by gas–liquid chromatography

Comparisons with average Danish food were made with values given by Helms (10) based on food analyses carried out by the Danish State Institute of Food

RESULTS

Table I lists the average amounts of the food components in caloric % for the seven subjects The corresponding values from other studies of arctic and Danish food are given for comparison

Table II presents the relative amounts (percentage of the total) of fatty acids in the fats of the food portions collected on seven days calculated as averages of all the specimens and subjects For comparison figures are given for common Danish food in 1972 (10)

Table III shows the sums of the contents of saturated monounsaturated and polyunsaturated fatty acids in the diet of Eskimos and Danes (10)

The daily intake of cholesterol for all seven Eskimos was on an average 245 mg/1 000 calories against an average of 139 mg/1 000 calories in Danish food (10)

Table II *Average content of fatty acids (percentage of the total fatty acids) in the food consumed by seven Eskimos during seven days and corresponding mean values for common Danish food*

Fatty acid	Eskimos x	S D	Danes x
C12 0	1 1	1 0	5 9
C14 0	5 7	1 1	7 5
C16 0	19 2	4 7	25 5
C16 1	13 5	4 5	3 8
C18 0	4 9	2 5	9 5
C18 1	29 7	5 3	29 2
C18 2	4 7	2 7	10 0
C18 3	0 4	0 3	2 0
C20 0	0 6	0 8	4 3
C20 1	6 9	3 5	0 4
C20 4	0 1	0 4	0
C20 5	2 3	1 4	0 4
C22 0	1 8	2 3	0
C22 1	4 6	2 9	1 2
C22 6	2 2	2 2	0 3
C24 0	0 4	0 8	0
C24 1	1 9	2 1	0

DISCUSSION

Several investigations have been published of the diet of arctic populations Table I surveys some major studies of Eskimo nutrition together with the results of the present study and—for comparison—the average composition of Danish food (10) Most of the studies deal however with the net composition of arctic food in terms of protein fat and carbohydrate and do not include the chemical composition in more detail No study has to our knowledge hitherto been carried out with the specific aim of examining the fatty acid composition of the dietary fats

Generally it has been found that the arctic populations consume more proteins (15) equal amounts of fats and less carbohydrates than the Western populations The high protein consumption has to do with the fact that meat—from seal and whale and to a lesser degree from fish and game—is the predominant nutriment of this population From hunting statistics from 1970 it was calculated that each subject in the district of Umanak consumed about 400 g seal and/or whale meat a day (2)

Table IV surveys the major nutriments for the seven persons under study during the period of food collection The average number of meals in which a given dietary component occurred during one week is recorded It is seen that whale and seal meat was eaten almost every day Sugar was used abundantly about five times a day mostly in coffee or tea

It was calculated from lists of goods sold in the shop run by the Royal Greenland Trading Company in the year of the present study (trading lists from the local store of the Trading Company in Igdlorssuit supplied by H Zeep) that the average daily consumption per capita in the settlement was 134 g bread biscuits and rye flour 31 g rice 42 g potatoes and 164 g sugar Using these figures to check our data it can be calculated that a carbohydrate intake of this amount corresponds to a daily caloric intake of 2832 kcal 37% of the calories were found to originate from carbohydrates This figure seems rather reasonable as an average

It is interesting that the fat intake of Eskimos was found to be similar to that of Danes or even lower considering the large meat/fish consumption and the relatively high fat content of seals and whales especially from the blubber and intestines parts of which are eaten However the large amount of fat consumed by Danes originates mostly from dairy products which are very scarce in the Eskimo diet

As already mentioned it is generally accepted that the fatty acid pattern which is ester-bound in the serum lipids reflects to some degree the fatty acid pattern of the dietary fats This relation was confirmed in the present study Even if the differences in the fatty acid composition of the plasma lipids between Eskimos and Danes did not exactly parallel those in the fatty acids in the food the overall patterns were similar This was most evident for palmitoleic (C16 1) and timnodonic (C20 5) acids which occurred more frequently in Eskimo foods and were found in larger amounts in all three plasma lipid fractions and for linoleic acid (C18 2) in which the differences were reversed (4)

The dietary intake of long chain polyunsaturated fatty acids—especially timnodonic (C20 5) and docosahexaenoic (C22 6) acids which are known to be present in marine fats—was found to be higher

Table III *Sums of the saturated monounsaturated and polyunsaturated fatty acids (percentage of total fatty acids) in Eskimo and Danish foods*

Fatty acids	*Eskimos*	*Danes*
Saturated	33 7	52 7
Monounsaturated	56 6	34 6
Polyunsaturated	9 7	12 7

Table IV *Average number of meals during one week, which contain one or several of the food components listed*

Whale (meat and blubber)	5 7
Seal (meat and blubber)	6 4
Seal (intestines)	0 6
Wildfowl	0 6
Fish	1 4
Soup with seal meat	2 3
Tinned food	0 7
Potatoes	1 9
Milk (powder)	0 7
Bread	13 6
Biscuits	1 0
Sugar	35 4

in Eskimos than in Danes, in whom it is negligible

The *sum* of the saturated and the *sum* of polyunsaturated fatty acids (Table III) in Eskimo foods were found to be smaller than in Danish diet, whilst the *sum* of the monounsaturated fatty acids was larger in Eskimo foods As the intake of polyunsaturated fatty acids of the C18 group was considerably lower in Eskimos (Table II) this means that the intake of other polyunsaturated acids must be higher The consumption of C20 5 and C22 6 seems important in this connection being 2 3 and 2 2%, respectively of the fatty acids consumed For comparison the intake of polyunsaturated acids in Danes except those originating from linoleic linolenic, and arachidonic acids is only 0 7% of the fatty acids consumed

As demonstrated by Keys et al (11) and confirmed by others (9) the level of serum cholesterol among other things a function of the dietary intake of polyunsaturated fatty acids relative to that of the saturated ones The influence of fatty acid intake on the serum cholesterol level is relative to the amount of saturated fatty acids minus half the amount of polyunsaturated fatty acids expressed as caloric % according to the following formula by Keys et al

$$\Delta\text{chol} = 2\,7\,(\Delta S - \Delta\, 1/2\, P) + 1\,5\,(\sqrt{C_2} - \sqrt{C_1})$$

where S and P are the caloric percentages of saturated and polyunsaturated fats respectively omitting stearic acid and C_1 and C_2 are mg cholesterol/1 000 calories in the two diets to be compared

Applying the formula to our data a change from Danish food (10) to the Eskimo food observed in this study, assuming an unchanged intake of calories would cause the serum cholesterol level to fall 7 6 mg/100 ml or 0 20 mmol/l This is not in accordance with the definitively lower serum cholesterol level found in Greenland Eskimos (?) compared with Danes of the same age (5) this difference ranging from 0 70 to 2 49 mmol/l in the various age groups with an average of 1 35 mmol/l

The higher content of long chain polyunsaturated fatty acids which was also observed both in the serum lipids and in the food of Eskimos indicates that these acids may be of major importance for the rather low serum cholesterol level in Eskimos Their action on the serum cholesterol level may differ qualitatively from that of the polyunsaturated fatty acids of the C18 group

At this point it may be important not to focus too strongly on the relationship between the low incidence of coronary occlusions among Eskimos and their low serum *cholesterol* level In fact the differences in serum *triglycerides* and *pre β-lipoprotein* between Eskimos and Danes were of a considerably higher *relative magnitude* than the differences in cholesterol and β lipoproteins (2) Generally the serum triglyceride and the pre β lipoprotein concentrations were lower than half of those of Danes

The possible influence of the food s composition on the serum triglyceride and pre β lipoprotein (very low density lipoprotein) levels is obscure However, it is probable that these low serum lipid values are related in some way to this composition too, and here one must consider the difference in carbohydrate intake However the amount and *composition of dietary fat in respect to its content* of polyunsaturated fats should also be carefully considered when assessing the plasma triglyceride and very low density lipoprotein concentrations Recently Grundy (8) called attention to the hypotriglyceridemic effect—together with the known hypocholesterolemic effect—of polyunsaturated fat and speculated that one of the main mechanism might be an effect on the metabolism of very low density lipoproteins as the reduction of cholesterol in his study was closely linked to that of triglycerides As mentioned earlier one of the most marked characteristics of the blood lipids in Eskimos was the very low concentration *of triglyceride* and very low density lipoproteins One can of course only speculate as to whether qualitative differences do exist between polyunsaturated fat in their ability to lower these substances but in our opinion it is a possibility that must be strongly considered

Although no epidemiological data so far have shown that high serum triglyceride (and pre β lipo protein) levels contribute information on the risk of coronary heart disease independently of the as sociated serum cholesterol level the very low levels of serum triglycerides and pre β lipoprotein in Eskimos may also be essential for their low inci dence of coronary occlusions and of diabetes mel litus

ACKNOWLEDGEMENT

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Peritoneal Dialysis in Hypernatraemic, Ketoacidotic Diabetic Coma

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ABSTRACT Hypertonic dehydration in a 13-year old boy with ketoacidotic diabetic coma has been treated successfully with peritoneal dialysis and *isotonic fluids Modes of treatment with either hypotonic or isotonic fluids are discussed, as is the feasibility of peritoneal dialysis We recommend isotonic solutions composed of equal parts of 5 5% glucose and 0 9% sodium chloride combined with peritoneal dialysis in order to secure a relatively slow correction of the hypertonic state*

Peritoneal dialysis has been used successfully by Finberg et al (7) in a special form of hypernatraemic dehydration i e accidental salt poisoning As osmolality is markedly increased in hypertonic dehydration it seems reasonable to equilibrate the body fluids against a solution which has a slightly higher osmolality than the internal milieu The peritoneum is suitable as an equilibration membrane and the dialysate can be adjusted to the desired osmolality

We have used peritoneal dialysis in ketoacidotic diabetic coma complicated with hypertonic dehydration The feasibility of peritoneal dialysis is discussed

CASE REPORT

The patient was a 13 year-old mulatto boy without known disposition to endocrine disorders Anorexia vomiting polydipsia and polyuria were present in the preceding two weeks A weight loss of 7 kg was registered On admission the patient was semicomatose with a smell of acetone The skin was warm and dry with normal turgor Pulse 136/min BP 130/100 mmHg temperature 36 8°C

Laboratory values on admission Hb 15 7 g/100 ml blood sugar 746 mg/100 ml serum sodium 174 mEq/l serum potassium 5 3 mEq/l standard bicarbonate 18 9 mEq/l blood urea 167 mg/100 ml urine for glucose +++ urine for acetone +++ Weight 45 kg Height 165 cm ECG normal

Treatment was started with crystalline insulin and 3 000 ml of a solution consisting of 100 mEq sodium 70 mEq chloride and 30 mEq lactate/1 000 ml and a supplement of 50 mEq potassium during the first 3 hours At this time blood sugar had been brought under control and peritoneal dialysis—with 2 l exchanges— with a dialysate containing glucose was considered permissible The composition of the dialysate was 130 mEq sodium 3 mEq potassium 4 5 mEq calcium 1 5 mEq magnesium 91 mEq chloride 45 mEq lactate and 75 mmol glucose/l i e 347 mosmol/l Peritoneal dialysis was continued for 14 hours with a total shift of 14 000 ml The total fluid deficit was judged to be about 7 000 ml and was repleted with isotonic solutions containing 77 mEq sodium 77 mEq chloride and 154 mmol glucose/1 000 ml i e 308 mosmol/l The derangement of body fluids was corrected over a period of 48 hours Fig 1 shows the values of serum sodium serum potassium osmolality and blood sugar during the first 64 hours of treatment

The osmolality was estimated using the formula

$$((Na^+ + K^+)\ mEq/l)\times 2+\frac{\text{blood sugar (mg/100 ml)}}{18}+\frac{\text{blood urea (mg/100 ml)}}{6}$$

A total of 292 U crystalline insulin was given during the first 24 hours

A neurological examination performed 18 hours after admission revealed no abnormalities except for a transient positive Babinski reflex The patient was discharged in good condition after one week

DISCUSSION

Hypertonic dehydration is a condition with a loss of water in excess of sodium As the extracellular volume generally is well preserved until late patients will be characterized by dry warm skin with normal turgor and no signs of circulatory disturbances (6)

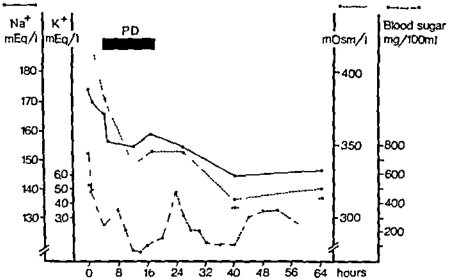


Fig 1 Serum values of sodium potassium osmolity and plasma glucose in the patient with ketoacidotic diabetic coma and hypertonic dehydration treated with peritoneal dialysis (P and isotonic solutions composed of equal osmoles of sodium chloride and glucose

In unregulated diabetes mellitus an adult loses about 6 l water 500 mEq sodium 400 mEq chloride and 350 mEq potassium per 24 hours from osmotic diuresis (1) In general the loss of water is largely compensated for by drinking and hyponatraemia supervenes However hypernatraemia is sometimes encountered (4)

Hypernatraemia per se can cause brain damage that is predominantly vascular as the resulting brain shrinkage leads to capillary dilatations with eventual rupture and interstitial haemorrhage (3 7) causing neurological disturbances and convulsions The immediate mortality attributable to the electrolyte disturbance in children is 10–15% and brain damage may be permanent in about 10% (10 13)

The seemingly logical treatment with hypotonic fluids is dangerous The brain capillaries allow free passage of water but not of solutes and as the brain is enclosed in a rigid cage it will function as a single cell and hypotonic solutions may cause brain swelling with disastrous effects on cerebral function (8) Furthermore some experimental results (12) suggest that the brain cells in response to dehydration are capable of manufacturing osmotically active substances idiogenic osmoles which may further accentuate brain oedema when hypotonic solutions are used

However several authors (4 9) recommend hypotonic solutions They consider isotonic solutions of sodium chloride potentially dangerous as the extracellular volume is expanded and the chloride may be responsible for an accentuation of a co-existing acidosis (11) Finberg (5) recommends a solution of 10% glucose in water to which sodium chloride and lactate have been added in concentrations of 75 50 and 25 mEq/l respectively This treatment requires a normal glucose metabolism The treatment in the present case comprises three phases 1) Initial treatment with hypotonic solutions 2) peritoneal dialysis with a slightly hypertonic dialysate (347 mosmol/l) to secure a slow correction of the hypertonicity and 3) repletion of body fluids with isotonic solutions with a low sodium content Treatment was successful as hypertonicity and dehydration were corrected within 48 hours without neurological deficits The diabetes mellitus caused no problems and serum potassium was easy to stabilize

The treatment outlined may have some disadvantages Hyperglycaemia and diabetic coma have been reported in diabetics treated with peritoneal dialysis when the dialysate contained glucose (2) Frequent determinations of blood sugar or use of sorbitol in the dialysate will prevent this complication The chloride content in the repletion solution may be too high and accentuate acidosis but this can be avoided by replacing sodium chloride with Ringer s lactate

In severe hypertonic dehydration we recommend rehydration with solutions composed of an equal mixture of 5 5% glucose and 0 9% sodium chloride combined with peritoneal dialysis until osmolality has reached a level of about 350 mosmol/l If the situation is complicated by unregulated diabetes mellitus the treatment must be initiated with hypotonic fluids until blood sugar is well under control

orrection of the abnormality must be relatively ow i e at least over 48 hours

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Blood Ketone Body Disappearance Rate in Diabetics and Normals after Rapid Infusion of DL-3-hydroxybutyrate

Studies before and after Diabetic Treatment

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ABSTRACT Ketone body tolerance has been studied in 26 newly diagnosed diabetics and 9 normal control persons after rapid i v infusion of DL-3 hydroxybutyrate In juvenile diabetics with high *initial fasting concentrations of ketone bodies* disappearance rates of acetoacetate and total ketone bodies in blood were low before diabetic regulation Insulin treatment normalized disappearance rates In the non obese maturity onset diabetics, on the other hand, ketone body disappearance rates remained abnormally low after treatment with glibenclamide In the obese maturity onset diabetics disappearance rates, being normal before diabetic regulation decreased during phenformin treatment, the rate constants becoming significantly lower than in the normals Decreased tissue uptake of ketone bodies thus seems to contribute to the increased ketone body level in blood in this group of diabetics Disappearance rates were not correlated to preinfusion ketone body concentration In the normals, no change in serum insulin was observed following the infusion while a significant decrease was seen in plasma glucose

In earlier studies (13) of the diurnal variation of blood ketone bodies in normal persons and in newly diagnosed diabetics it was found that blood ketone body level was nearly normalized during antidiabetic treatment in the juvenile and the non obese maturity onset diabetics while it increased during phenformin treatment in the obese maturity onset diabetics

In the present *investigation the kinetic response* of ketone bodies in blood to rapid i v administration of DL 3 hydroxybutyrate was studied in diabetics and non diabetic controls to examine whether changes of ketone body disappearance rate reflecting tissue uptake of ketone bodies are involved in the above mentioned changes in blood ketone bodies The response in serum insulin concentration to infusion of DL 3 hydroxybutyrate was examined simultaneously in the non-diabetics

MATERIAL AND METHOD

The study was performed in 26 newly diagnosed diabetics and 9 non-obese control persons without diabetes The diabetic group consisted of 10 non-obese juvenile diabetics (aged *14–55* years mean *28*) 9 non-obese maturity onset diabetics (aged 38–77 years mean 60) and 7 obese maturity onset diabetics (aged 47–77 years mean 64) Mean degree of overweight (8) in the obese maturity onset diabetics was 48% (range 26–73) The controls (aged 20–38 years mean 26) had normal oral glucose tolerance test no family history of diabetes and were admitted to hospital because of minor diseases of the locomotor system or neurotic disorders

Treatment

The juvenile diabetics and the non-obese maturity onset diabetics were given a diet containing about 2000 kcal (100 g protein 190 g carbohydrate and 85 g lipid) together with insulin or glibenclamide respectively Insulin 4–16 U was given in the morning and two diabetics in the afternoon as well All the juvenile diabetics received isophane insulin and three regular (crystalline) insulin also The daily dose of glibenclamide was 2 1/2–20 mg The obese maturity onset diabetics were treated with a *1200 kcal diet (105 g protein 105 g carbohydrate and 40 g* lipid) together with phenformin 50–100 mg daily

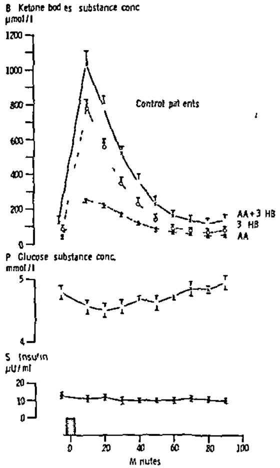


Fig 1 Blood ketone bodies plasma glucose and serum insulin after rapid infusion of DL 3 hydroxybutyrate in 9 controls (mean±S E M) every 10 min for each substance Ø=infusion time *AA*=acetoacetate *3 HB*=3 hydroxy butyrate

Experimental procedure

On the day of admission controls as well as diabetics were given the 2000 kcal diet At 8 a m on the next day when the patients had fasted for 12 h and before administration of insulin or oral antidiabetic drugs 50 ml of sodium DL 3 hydroxybutyrate 1 mol/l was injected i v over 4 min A 5 ml blood sample was taken from an indwelling catheter inserted into an antecubital vein just before injection and every 10 min during the following 90 min from the middle of the injection Urine was collected for the 90 min study period In the diabetics the studies were repeated after the best possible diabetic regulation had been obtained Insulin or oral antidiabetics were given at least 14 h before the investigation In three juvenile diabetics treatment had to be started just after admission so that studies were performed only after diabetic regulation

Ketonuria could not be detected after diabetic regulation using Acetest® In the juvenile diabetics plasma glucose substance concentration varied during the day between 5 3 and 14 0 mmol/l and the 24-hour urinary excretion of glucose was below 140 mmol In the maturity onset diabetics plasma glucose was below 10 5 mmol/l throughout the day and the urine practically glucose free

The concentrations of acetoacetate (AA) and 3 hydroxybutyrate (3 HB) in the blood samples and urines were determined by an enzymatic micromethod (12) Total ketone bodies (AA+3 HB) were calculated by summation of the concentrations of AA and 3-HB Glucose in plasma and urine was measured by an o-toluidine method (6) Serum insulin was analysed with a radioimmunoassay technique employing wick chromatography (4)

The Wilcoxon test for paired differences the Wilcoxon test for two samples and Spearman s rank correlation were used in the statistical analysis

Calculation of ketone body disappearance rate

The concentrations of 3 HB and AA+3 HB in blood increased rapidly after the injection of DL 3 hydroxybutyrate the highest concentrations being measured 10 min postinfusion while the concentration of AA in blood rose more slowly (Figs 1 and 2) Disappearance rate constants (K) in blood for excess AA 3 HB and AA+3 HB (i e the substance concentrations of AA 3 HB and AA+3-HB in excess of the concentrations in blood before injection of sodium DL 3 hydroxybutyrate) were calculated in principle as described by Owen et al (9) In all persons investigated excess substance concentrations of 3 HB and AA+3 HB decreased exponentially with time from 20 min postinfusion while excess substance concentrations of AA in some persons reached maximum later on and then decreased exponentially i e plotting ketone body substance concentration excesses against time in a semilogarithmic system resulted in a straight line (Fig 3) The linear regressions of the lines were calculated using least squares analysis (the coefficient of correlation being ≥0 87) Ketone body disappearance rate K was defined as the slope of the line and in each study calculated for AA (K_{AA}) 3 HB ($K_{3\text{-}HB}$) and AA+3-HB ($K_{AA+3\text{-}HB}$) However in two juvenile diabetics (nos 1? and 15) $K_{3\,HB}$ was impossible to calculate before treatment the disappearance rate being influenced by rapid excretion of 3 HB in the urine

RESULTS

Table I shows the amount of substance of ketone bodies excreted in the urine during the studies preinfusion substance concentrations of plasma glucose and blood ketone bodies as well as ketone body disappearance rate constants in blood (K) for the diabetics and controls

Preinfusion ketone body concentration in blood

Before diabetic treatment the mean preinfusion total ketone body concentration in blood was significantly higher in all groups of diabetics than in the controls ($p<0\ 05$) After diabetic regulation this

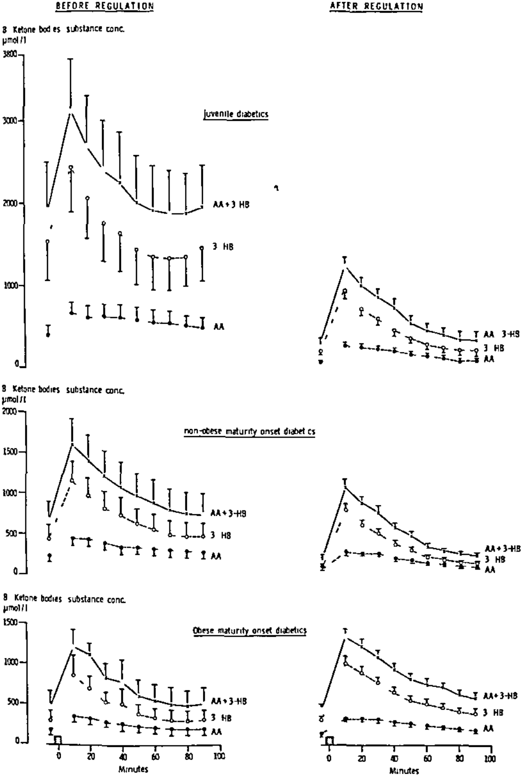


Fig 2. Blood ketone bodies after rapid infusion of DL 3 hydroxybutyrate in juvenile diabetics, non-obese maturity onset diabetics and obese maturity onset diabetics before and after diabetic regulation (mean ±S.E.M.) every 10 min for each substance. Symbols as in Fig. 1.

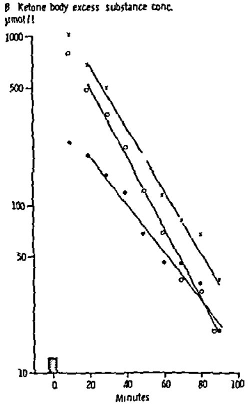


Fig 3 Blood ketone body excess plotted semilogarithmically versus time after infusion of DL 3 hydroxybutyrate in control patient 3 ▢=infusion time ●=acetoacetate O=3 hydroxybutyrate ×=acetoacetate+3 hydroxybutyrate

oncentration had decreased markedly in the juvenile and the non-obese maturity onset diabetics but was still significantly higher in the juvenile diabetics than in the controls ($p<0\ 01$) In the obese maturity onset diabetics blood ketone bodies increased during diabetic regulation in 6 patients and decreased in 1 patient the mean concentration being nearly unchanged and still significantly higher than in the controls ($p<0\ 01$)

Urinary excretion of ketone bodies

In the controls the juvenile diabetics after diabetic regulation and in the maturity onset diabetics the mean urinary ketone body excretion during the study periods of 90 min varied between 3 4 and 5 2% of the amount of D-3 hydroxybutyrate infused and did not differ significantly between these groups of diabetics and the controls ($p>0\ 05$) In the juvenile diabetics before diabetic regulation the mean urinary ketone body excretion was 29 8% of the amount infused significantly higher than in the controls ($p<0\ 01$)

Ketone body disappearance rate in blood

Controls The exogenously derived ketone bodies disappeared rapidly from the blood as reflected by a mean total ketone body disappearance rate constant of $6\ 6\times10^{-2}$ min^{-1} The highest mean disappearance rate was found for 3 HB $K_{3\text{-}HB}$ being significantly different from $K_{AA+3\text{-}HB}$ ($p<0\ 05$) In each control person K_{AA} was lower than both $K_{3\text{-}HB}$ and $K_{AA+3\text{-}HB}$

Diabetics Before diabetic regulation mean disappearance rates of ketone bodies in the juvenile diabetics were significantly influenced by the high urinary excretion of ketone bodies during the study periods and comparison with mean disappearance rates of ketone bodies in the controls was not possible In the non-obese maturity onset diabetics mean disappearance rates of AA 3 HB and AA+3 HB were significantly lower than in the controls ($p<0\ 05$) while no significant differences were found between controls and obese maturity onset diabetics ($p>0\ 05$)

After diabetic regulation no significant differences were found between the disappearance rates of ketone bodies for controls and juvenile diabetics In the non obese maturity onset diabetics mean disappearance rates of 3 HB and AA+3 HB were still significantly lower than in the controls ($p<0\ 05$) In the obese maturity onset diabetics mean disappearance rates of AA 3 HB and AA+3 HB decreased during diabetic treatment and were significantly lower than in the controls ($p<0\ 01$)

Antidiabetic treatment did not change disappearance rates of AA 3 HB and AA+3 HB significantly in the non-obese maturity onset diabetics ($p>0\ 05$) whereas a significant decrease was found in the obese maturity onset diabetics ($p\leq0\ 05$)

Serum insulin and plasma glucose

Fig 1 presents the temporal relationships of serum insulin plasma glucose and blood ketone bodies to DL 3 hydroxybutyrate infusion in the controls Plasma glucose was significantly depressed 20 min postinfusion compared with initial values ($p<0\ 01$) The decrease in serum insulin was slight and non significant

In the diabetics the preinfusion concentration of

I *Postinfusion urinary and preinfusion blood ketone bodies preinfusion plasma glucose and ketone disappearance rate constants in blood (K)*

at	90 min postinfusion excretion of U ketone bodies (AA+3 HB)		Preinfusion concentration (mmol/l)				K (10 ^{2}min^{-1})		
	mmol	%	P glucose	B AA	B 3 HB	B-(AA+3 HB)	B AA	B 3 HB	B (AA+3 HB)
ontrols									
1	0 84	3 4	5 4	0 036	0 041	0 077	4 6	7 4	5 9
2	0 98	3 9	4 8	0 091	0 291	0 382	8 3	10 9	11 7
3	0 17	0 7	4 4	0 014	0 005	0 019	3 3	4 9	4 3
4	0 61	2 4	4 8	0 018	0 018	0 036	4 8	5 5	6 1
5	2 28	9 1	4 9	0 050	0 114	0 164	4 6	8 6	6 1
6	0 87	3 5	5 2	0 014	0 005	0 019	4 6	8 6	5 7
7	1 29	5 2	4 8	0 100	0 159	0 259	4 4	8 2	6 6
8	0 15	0 6	4 3	0 027	0 005	0 032	7 2	10 2	8 0
9	1 78	7 1	4 7	0 041	0 064	0 105	4 0	6 3	5 3
Mean		4 0	4 8	0 043	0 078	0 121	5 1	7 8	6 6
±S E M		0 9	0 1	0 011	0 032	0 042	0 5	0 7	0 7
Juvenile diabetics before treatment									
10	1 91	7 6	13 1	0 109	0 450	0 559	6 1	5 5	10 8
11	5 10	20 4	15 0	0 237	0 787	1 024	2 6	6 1	3 6
12	8 52	34 1	14 5	0 787	2 948	3 735	2 5	–	6 4
13	15 99	64 0	10 0	0 496	2 139	2 635	1 4	4 7	2 9
14	1 27	5 1	11 6	0 205	0 364	0 569	10 8	12 8	9 5
15	16 54	66 0	14 0	0 792	3 230	4 022	2 1	–	5 1
16	2 89	11 6	15 2	0 228	0 728	0 956	1 1	12 7	3 5
Mean		29 8	13 3	0 408	1 521	1 929	3 8	8 4	6 0
±S E M		9 8	0 7	0 108	0 462	0 570	1 3	1 8	1 2
Juvenile diabetics after treatment									
10	0 87	3 5	5 9	0 082	0 096	0 178	6 5	7 4	6 3
11	2 25	9 0	12 0	0 146	0 346	0 492	4 1	6 0	4 9
12	0 33	1 3	10 1	0 082	0 118	0 200	6 2	11 6	8 2
13	2 04	8 2	9 0	0 046	0 378	0 424	1 4	5 3	4 2
14	0 79	3 2	8 0	0 086	0 137	0 223	4 5	8 1	8 1
15	0 94	3 8	14 2	0 096	0 214	0 310	3 7	5 8	6 1
16	1 18	4 7	7 6	0 096	0 218	0 314	6 1	4 6	5 9
17	1 63	6 5	11 0	0 073	0 146	0 219	1 6	2 6	2 2
18	1 46	5 8	10 2	0 050	0 091	0 141	3 4	5 0	5 0
19	0 42	1 7	5 3	0 182	0 532	0 714	1 9	2 4	2 0
Mean		4 8	9 3	0 094	0 228	0 322	3 9	5 9	5 3
±S E M		0 8	0 9	0 013	0 046	0 056	0 6	0 9	0 7
Non obese maturity onset diabetics before treatment									
20	1 05	4 2	13 1	0 218	0 628	0 846	2 0	7 5	6 9
21	2 29	9 1	16 0	0 250	0 496	0 746	2 4	6 3	4 4
22	0 70	2 8	11 1	0 123	0 086	0 209	5 9	3 5	4 4
23	2 64	10 6	14 8	0 555	1 502	2 057	1 4	2 3	1 5
24	0 33	1 3	9 3	0 014	0 014	0 028	1 9	4 6	3 1
25	0 26	1 0	15 4	0 400	0 346	0 746	3 0	3 0	2 8
26	3 33	13 3	8 9	0 391	0 878	1 269	6 1	9 7	5 8
27	0 18	0 7	13 6	0 041	0 064	0 105	2 1	3 4	2 9
28	0 47	1 9	9 6	0 064	0 072	0 136	4 2	6 6	5 7
Mean		5 0	12 4	0 228	0 455	0 682	3 2	5 2	4 2
±S E M		1 6	0 9	0 063	0 164	0 222	0 6	0 8	0 6

Table I *(Continued)*

Pat no	90 min postinfusion excretion of U ketone bodies (AA+3 HB) mmol	%	Preinfusion concentration (mmol/l) P glucose	B AA	B 3 HB	B-(AA+3 HB)	K (10^{-3} min^{-1}) B AA	B 3 HB	B-(AA+3 HB)
Non obese maturity onset diabetics after treatment									
20	0 88	3 5	6 8	0 096	0 100	0 196	4 1	4 0	4 4
21	1 38	5 5	12 1	0 100	0 109	0 209	4 0	5 1	4 5
22	0 42	1 7	6 5	0 046	0 032	0 078	5 0	3 5	3 6
23	0 56	2 2	8 2	0 023	0 046	0 069	2 1	3 0	[illegible]7
24	0 37	1 5	7 6	0 027	0 023	0 050	4 2	3 1	3 2
25	1 87	7 5	5 2	0 173	0 182	0 355	5 6	7 1	7 7
26	1 02	4 1	3 6	0 109	0 146	0 255	6 0	4 4	5 8
27	0 05	0 2	6 2	0 046	0 055	0 101	4 0	4 0	3 8
28	1 02	4 1	7 3	0 177	0 328	0 505	4 0	6 6	5 8
Mean		3 4	7 1	0 089	0 113	0 202	4 3	4 5	4 6
±S E M		0 8	0 8	0 020	0 032	0 051	0 4	0 5	0 5
Obese maturity onset diabetics before treatment									
29	0 95	3 8	11 4	0 159	0 268	0 427	4 7	5 8	4 8
30	1 43	5 7	11 5	0 205	0 445	0 650	6 4	9 9	11 5
31	0 93	3 8	16 8	0 073	0 114	0 187	3 2	3 6	3 4
32	0 98	3 9	12 6	0 105	0 173	0 278	4 4	6 7	6 1
33	0 87	3 5	13 2	0 105	0 059	0 164	4 8	3 4	4[illegible]
34	3 54	14 2	12 8	0 501	1 030	1 531	3 0	8 5	5 7
35	0 32	1 3	11 3	0 059	0 023	0 082	3 1	3 4	4 0
Mean		5 2	12 8	0 172	0 302	0 474	4 2	5 9	5 7
±S E M		1 3	0 7	0 058	0 133	0 191	0 5	1 0	1 0
Obese maturity onset diabetics after treatment									
29	1 05	4 2	7 6	0 159	0 382	0 541	2 7	2 8	2 2
30	0 47	1 9	8 7	0 191	0 491	0 682	3 6	5 6	4 9
31	1 43	5 7	8 3	0 159	0 373	0 532	1 8	2 3	1 7
32	1 03	4 1	8 4	0 145	0 291	0 436	4 2	5 2	5 0
3	0 98	3 9	9 5	0 123	0 268	0 391	2 5	3 0	2 8
	0 51	2 0	8 1	0 077	0 255	0 332	3 3	6 3	[illegible]0
5)	1 62	6 5	8 1	0 086	0 187	0 273	1 3	2 4	1 9
lean		4 0	8 4	0 134	0 321	0 455	2 8	3 9	3 4
±S E M		0 7	0 2	0 016	0 038	0 053	0 4	0 6	0 6

glucose in plasma before treatment was above 8 3 mmol/l In all groups of diabetics there were only small fluctuations in plasma glucose during the study periods with a slight decrease in mean concentration

Relationship between ketone body disappearance rate in blood and blood ketone bodies

There was no significant correlation ($p>0\ 05$) between the preinfusion concentrations of AA 3 HB and total ketone bodies in blood and the respective disappearance rate constants

DISCUSSION

Apart from the majority of the juvenile diabetics before treatment the urinary excretion of ketone bodies during the study periods did not influence ketone body disappearance rates significantly Thus in the controls in the juvenile diabetics during diabetic regulation and in the maturity onset diabetics ketone body disappearance rates reflected tissue uptake of ketone bodies However disappearance rates of AA and 3 HB are influenced by the formation of AA from infused DL 3-hy

ybutyrate according to the reaction 3 HB+ D⁺⇌AA+NADH+H⁺ Therefore the disappearance rate of total ketone bodies ($K_{AA+3\text{-}HB}$) gives the most correct estimate of tissue uptake of ketone bodies allowing a quantitative estimate of the rate of ketone body utilization

In five of the juvenile diabetics with preinfusion ketone body levels of 1–4 mmol/l K_{AA} and $K_{AA+3\text{-}HB}$ were low in spite of high urinary excretion of ketone bodies (mainly 3 HB) In two patients of this group with only slightly increased preinfusion ketone body concentrations of about 0 5 mmol/l and a normal urinary excretion of ketone bodies during the studies disappearance rates were normal or high Thus in juvenile diabetics with severe ketosis tissue uptake of ketone bodies seems to be low while in juvenile diabetics with slight ketosis the tissue uptake is normal or increased Diabetic regulation normalized disappearance rates

The results are in agreement with the experiments of Bassler et al (4) showing decreased utilization of ketone bodies in alloxan diabetic rats when ketone body production was high while no disturbance was seen at low production rates In these studies ketone body utilization was normalized during insulin treatment Evidence of impairment in the oxidation of AA has also been found in diabetic rats (3) and it is likely that peripheral utilization approaches maximum at some given ketone body concentration beyond which small differences in production result in major differences in total ketone body concentration in blood (2 7)

In the non-obese maturity onset diabetics treatment with glibenclamide did not restore $K_{3\text{-}HB}$ and $K_{AA\ 3\text{-}HB}$ the ketone body metabolism remaining abnormal

During phenformin treatment of obese maturity onset diabetics we have earlier found that the blood ketone body level increased in spite of satisfactory diabetic regulation judged from glucose measurements (13) In the present study the fasting concentration of ketone bodies in blood also increased during phenformin treatment in nearly all the patients simultaneously with a significant fall in ketone body disappearance rates Decreased tissue uptake suggesting decreased utilization thus seems to take part in the ketosis of phenformin treated diabetics

It is generally presumed that tissue uptake of ketone bodies is a function of ketone body concentration in the blood (10) The lack of a correlation in this study between ketone body disappearance rates and the preinfusion ketone body concentration in blood indicates that other factors than the ketone body concentration determine tissue uptake at least in diabetics

Previous studies on the insulinogenic effect of ketone bodies in man have been controversial (1 5 9 11) In the present study there was no change in serum insulin concentration after a rise in blood ketone bodies within physiological limits and changes in serum insulin thus did not interfere with the ketone body disappearance rate in blood The reported differences in human studies can be explained by differences in the amount of ketone body infused and as pointed out by Owen et al (9) by the length of the interval between ketone body administration and blood sampling The decreasing concentration of plasma glucose after administration of ketone bodies also seen in this study has been found to be a consequence of a reduction in hepatic glucose output (1)

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Liver Enzymes in Alcohol-discordant Twins

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ABSTRACT Seventy moderately alcohol discordant male twin pairs have been investigated with respect to some liver enzymes No differences were found as regards S ALAT, while significantly higher enzyme levels were demonstrated for S-ASAT, S-ALP and S GT among the high alcohol consumers compared to their low consuming co-twins Especially S-GT was found to be a valuable and sensitive test in the detection of even a moderate alcohol intake in working and socially well adapted subjects These results were obtained in a subject group in which the influence of genetic factors was kept to a minimum

S ALAT (serum alanine aminotransferase formerly called S GPT) and S ASAT (serum aspartate aminotransferase formerly called S-GOT) are considered to be valuable parameters in liver disease As alcohol—in this context ethanol—is a hepatotoxic substance raised liver enzymes are usually found in alcoholics S ASAT as a rule exceeding S ALAT after an acute debauch The enzyme γ glutamyl transpeptidase (S-GT) has attracted growing interest in recent years as—compared to either the transferases or S ALP (serum alkaline phosphatase)—it has proved to be a more sensitive parameter in drinkers and alcoholics when the clinical diagnosis was not known initially (10)

S-GT is present in normal serum and raised serum levels are found in hepatobiliary and pancreatic diseases (13 15) Rosalki et al (12) and Rosalki and Rau (11) have reported a high frequency of elevated S GT levels in patients with a clinical diagnosis of alcoholism moreover S-GT was the only enzyme raised in the group classified as heavy drinkers rather than alcoholics

The aim of the present study was to investigate possible effects of a moderate long standing intake of alcohol on some liver enzymes The subject group comprised moderately alcohol discordant twin pairs thus reducing the influence of genetic factors and affording favourable possibilities of studying the effect of alcohol even at small differences in consumption

MATERIAL AND METHODS

The group of subjects was assembled from the Swedish Twin Registry (5 6) At the time of the present study the registry comprised about 10000 sets of same sexed twins covering about 95% of all Swedish same sexed twins born in the country in 1886–1925 and still alive at the time of the compilation Data concerning the twin pairs were collected mainly by means of three questionnaires These yielded information on alcohol consumption and on basis of this 92 alcohol-discordant male twin pairs aged 45–65 years were invited to participate in the present study Seventy complete pairs were examined The criterion for discordance was set at >10000 versus<2000 g absolute alcohol per year (a bottle of beer (33 cl)=12 g a bottle of wine (75 cl)=60 g a bottle of spirits (75 cl)=250 g absolute alcohol) The validity of the amounts of alcohol consumed has been confirmed and reported by Myrhed (8) The moderate level of consumption even among the high consumers is demonstrated by their monthly average which was less than 1300 g absolute alcohol

Blood samples were taken from the subjects in the morning following an overnight fast Serum was obtained by centrifugation and S ALAT S ASAT and A ALP were determined for all subjects Twin pairs discordant as to >1000 g absolute alcohol/month were also analysed subsequently and at the same time with regard to S-GT Only one of the subjects investigated had detectable alcohol in blood Zygosity was determined according to serologic determination of 8 blood group systems and 11 serum groups as described by Myrhed (8) Out of the 70 twin pairs 14 turned out to be monozygotic (MZ) and 56 dizygotic (DZ)

S ALAT and S ASAT were analysed using a reaction rate analyser (LKB 8600) connected to an evaluation unit (Optilab Bo Philip Instrumentation Stockholm) Reagents for S ALAT and S-ASAT were obtained from Kabi Stockholm Reference values ≤35 U/l

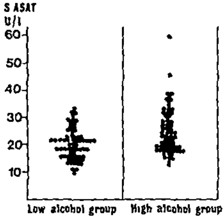


Fig 1 Serum aspartate aminotransferase (S ASAT) activities in alcohol discordant twin pairs pooled group (MZ+DZ)

S ALP was analysed according to Bergström and Thunblad (2) Reference value ≤35 U/l

S GT was analysed according to Szasz (14) Reagents were obtained from Roche Diagnostica Basel Reference value ≤40 U/l

The mean intrapair differences between high and low alcohol consumers were evaluated with Student s paired *t* test

RESULTS

S ASAT

The effect of alcohol on the S ASAT levels is obvious as seen in Fig 1 and Tables I and II In the pooled zygosity group (MZ+DZ) mean values were 22 6 and 19 2 U/l in the HAG (high alcohol group) and LAG (low alcohol group) respectively ($p<0\ 001$) *The tendency is the same in the MZ as* well as in the DZ group The difference between means is more accentuated when confined to twin pairs discordant with respect to >1000 g absolute alcohol/month (Table II) Here the mean values are 25 1 and 19 4 for HAG and LAG, respectively ($p<0\ 01$)

Another way to illustrate the difference within pairs is to assess the values in a qualitative manner i e to see which of the twins within a pair had the higher S ASAT value Following this procedure in *the pooled zygosity group 41 of 66 pairs in HAG* have higher enzyme values (Table II) In the highly discordant group the corresponding figures are 17 of 23

S ALAT

No obvious differences were found in the pooled zygosity group with respect to S ALAT (Tables I and II) The mean values were 18 6 and 17 6 U/l in HAG and LAG, respectively The difference between the means is somewhat greater—though still not significant—in the twin pairs discordant *at >1000 g absolute alcohol/month* (20 3 *versus* 17 1 U/l)

S ALP

Even a moderate alcohol intake affects the level of S ALP Thus mean values in the pooled zygosity group amounted to 23 1 and 20 8 U/l (Tables I and II) for HAG and LAG respectively ($p<0\ 05$) The difference between means was more pronounced for the twin pairs discordant with respect to >1000 g absolute alcohol/month, the means being 25 7 U/l *for HAG and 21 3 U/l for LAG* ($p<0\ 05$)

On a qualitative basis the high consumers had the higher S ALP value in 37 of 64 pairs in the pooled zygosity group (Table II) The opposite i e the low consumer within a pair had the higher enzyme value was true in 22 pairs In the group discordant at *>1000 g/month the high consumer in 17 of 23 pairs* had the higher S ALP level

S GT

The greatest disparities between mean values were found for S GT However only twin pairs discordant with respect to >1000 g absolute alco-

Table I *Serum enzymes (U/l) in relation to alcohol consumption (mean values S D within parentheses)*

	MZ			DZ			MZ+DZ		
	HAG	LAG	*No of* pairs	HAG	LAG	*No of* pairs	HAG	LAG	No of pairs
S ASAT	19 3 (5 4)	18 7 (4 6)	14	23 4 (8 0)	19 4 (4 2)***	52	22 6 (7 7)	19 2 (4 3)***	66
S-ALAT	13 7 (3 7)	16 3 (8 0)	14	19 8 (10 7)	18 0 (8 9)	52	18 6 (10 0)	17 6 (8 7)	66
S ALP	20 5 (9 9)	18 1 (6 6)	14	23 8 (8 6)	21 7 (7 6)	51	23 1 (8 9)	20 8 (7 5)*	65

* $p<0\ 05$ *** $p<0\ 001$

Table II *Qualitative evaluation of S ASAT S ALAT S ALP (U/l) in relation to alcohol consumption*

	MZ+DZ			MZ+DZ >1 000 g abs alc /month		
	HAG	LAG	No of pairs	HAG	LAG	No of pairs
Higher S ASAT value	41	22	66	17	6	23
Mean	22 6	19 2		25 1	19 4	
S D	7 7	4 3***		9 5	5 0**	
Higher S ALAT value	29	31	66	11	10	23
Mean	18 6	17 6		20 3	17 1	
S D	10 0	8 7		11 5	6 9	
Higher S ALP value	37	22	65	17	5	22
Mean	23 1	20 8		25 7	21 3	
S D	8 9	7 5*		9 3	7 3*	

*p<0 05 **p<0 01 ***p<0 001

hol/month were analysed for this enzyme (Fig 2 and Table III) In the pooled zygosity group 18 of 22 pairs in HAG displayed the higher enzyme value Mean values for HAG and LAG were 32 6 and 11 0 U/l respectively ($p<0$ 01) In all the 5 MZ pairs it *was the high consumer who had the higher enzyme* value Means amounted to 40 8 and 10 4 U/l for HAG and LAG respectively The same tendency was found in the DZ group in which the corresponding values were 30 2 and 11 1 U/l ($p<0$ 05)

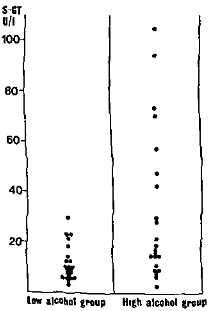


Fig 2 Serum γ glutamyl transpeptidase (S-G T) activities in alcohol-discordant twin pairs discordance>1 000 g abs alc /month pooled group (MZ+DZ)

DISCUSSION

This intrapair comparison in moderately alcohol discordant twin pairs has revealed a significant disparity with regard to S ASAT S ALP and S GT No obvious difference was found with respect to S ALAT However the enzyme values were within normal limits in both groups Despite the highly significant difference for S ASAT only two of the high consumers had pathological serum values i e exceeding 35 U/l indicating that liver damage would scarcely have been suspected in any of the subjects in an ordinary medical examination Similar results were found with respect to S ALAT and S ALP The present study suggests that long standing moderate alcohol consumption does not necessarily give rise to any serious enzyme leakage caused by cell necrosis

S ASAT seems to be the most sensitive of the transferases as no obvious differences were found for S ALAT This is in accordance with Bang et al (1) who found a rise in S ASAT after acute alcohol intoxication in 27 of 35 alcoholic patients Brohult et al (3) were able to show that a single dose of alcohol corresponding to 3 g/kg b wt administered to apparently healthy men produced a significant rise in S ASAT for a few days after its intake In another study Konttinen et al (7) found that S ASAT was abnormal in alcoholics more often than the other liver enzymes As increased serum creatine kinase activity was found among 43% of the patients it was concluded that S ASAT was released to some extent from heart or skeletal muscles In order to show more specific liver damage the authors suggested the use of serum ornithine

Table III *S GT (U/l) in relation to alcohol consumption at discordance >1 000 g absolute alcohol/month*

	MZ			DZ			MZ+DZ		
	HAG	LAG	No of pairs	HAG	LAG	No of pairs	HAG	LAG	No of pairs
Higher S-GT value	5	0	5	13	4	17	18	4	22
Mean	40 8	10 4		30 2	11 1		32 6	11 0	
S D	39 0	2 7		27 5	7 9*		29 8	7 0**	

* $p<0\ 05$ **$p<0\ 01$

carbamoyl transferase (S OCT) and S GT Concerning the small differences in S ALAT it should be noted that in the present twin study as well as in the other studies mentioned above the interval since the most recent intake of alcohol was rather short Wallgren and Barry (16) have suggested that the rise of S ALAT is more pronounced a few days after the imbibing of alcohol has stopped while at *the same time there is a fall of S ASAT*

Although the group means for S ALP in the present study did not differ markedly intrapair testing revealed significant disparities between high and low consumers Elevated S ALP values have been reported in alcoholics (16) Out of 100 chronic alcoholics with a history of heavy drinking 23 had pathological S ALP values (7) but the group included at least twice that number of subjects with pathological transferase levels Rosalki and Rau 11) in a study of anicteric alcoholics and heavy nkers found only 5 4% with pathological S ALP alues and concluded that this enzyme is of little value in the detection of early liver damage In a study of 16 chronic alcoholics Brohult and Sundblad (4) *demonstrated abnormal agar gel electro*phoretic patterns of alkaline phosphatase isoenzymes in 11 in spite of normal or borderline routine tests including S ALP S ASAT and S ALAT The abnormality consisted of the presence of a fast moving α fraction which was considered to be a more sensitive indicator than S OCT and lactate dehydrogenase isoenzymes (S LDH) This illustrates the advantage of the co twin control method over the studies mentioned above—although there were few pathological values it is obvious that even rather small amounts of alcohol influence the S ALP level

Analysis of S-GT in the highly discordant group produced the most striking disparities Thus in all *but 4 of the 22 pairs the high consumer had a higher* S-GT value than the low consuming co-twin This is notable as the high consumers had a fairly moderate intake of alcohol and comprised subjects who were working and socially well adapted Earlier studies have focused mainly on alcoholics and heavy drinkers In the study of Konttinen et al (7) S ASAT S OCT and S GT were the enzymes most often found to be pathologic in alcoholics Rosalki and Rau (11) showed that the S-GT activity was *raised in some three-quarters of alcoholics or heavy* drinkers while transferase elevation was observed in less than one third S GT in this study was the only enzyme to be elevated in the group classified as heavy drinkers rather than alcoholics Rollason et al (9) determined S GT in 5 groups of individuals imbibing differing amounts of alcohol In the group with the heaviest consumption 46 9% displayed abnormal S-GT values compared to 21 6% among the teetotallers S GT was also found to give the best measure of the effects of alcohol compared to S ASAT and S ALP Concerning specificity transferase or alkaline phosphatase activity may be released from various organs while increased S-GT activity is almost invariably a result of hepatobiliary *or pancreatic disease (7 10 11) In the present* study it seems unlikely that the raised S-GT was of pancreatic origin since none of the subjects had clinical evidence of pancreatic disease It is therefore considered that besides being easy to assess the determination of S GT is more sensitive than other enzyme analyses for the detection of liver cell damage after moderate alcohol intake

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Reduction of Isoniazid Bioavailability in Normal Men by Concomitant Intake of Food

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ABSTRACT The influence of food intake on the bioavailability of isoniazid (INH) has been examined in nine healthy male volunteers INH was administered as a single oral dose both in fasting state and together with a standardized breakfast Numerous venous blood samples were obtained 5 min–6 hours after the INH ingestion, and the concentrations of unmetabolized INH in serum were assessed by spectrophotometry The observations indicate that both the peak concentration and the total amount of INH absorbed are greatly reduced when the drug is ingested together with food Hence it is recommended that in the treatment of tuberculosis with INH, the drug should be given on an empty stomach The data may also have some bearing on the use of INH for assessing acetylation rates and estimating dosages of hydralazine and related drugs

Among patients and physicians alike a common question regarding oral administration of drugs is whether the tablet(s) should be taken on an empty stomach or together with food This question often remains unanswered as information about the influence of food intake on the gastrointestinal absorption of drugs is available only exceptionally In an effort to obtain such information we have studied the bioavailability of different drugs after single dose administration both on a fasting stomach and together with a standardized meal

Isoniazid (INH) was included in the studies for several reasons In addition to the fact that digestion induced changes in gastric emptying and gastrointestinal motility and blood flow might alter the absorbed quantity of many drugs INH is a highly reactive compound which may be easily affected both by various food components and by food induced changes in the gastrointestinal acid–base balance Indeed antacid drugs have been shown to reduce the absorption of INH (3) Nevertheless it is recommended at least in this country that in the treatment of tuberculosis the single daily dose of INH should be taken together with a meal Apart from its use as a tuberculostatic drug INH is increasingly employed as a test substance in the phenotyping of acetylation capacity but there is no general agreement as to whether in this test the drug should be taken before or together with the breakfast

The present study indicates that both the peak concentration and the total amount of INH absorbed are greatly reduced when the drug is ingested together with food Thus in the treatment of tuberculosis with INH the drug should be given on an empty stomach The data may also have some bearing on the use of INH for assessing acetylation rates and estimating dosages of hydralazine and related drugs

MATERIAL AND METHODS

Nine clinically healthy male volunteers aged 28–35 weight range 67–77 kg served as test subjects Liver function as judged by assessments of transaminase and bilirubin levels in blood was normal in all After total abstention from food and liquid for ten hours (9 p m –7 a m) a polyethene cannula was inserted into an antebrachial vein and 10 ml of blood was collected (0 value blank) Thereafter INH—100 mg/10 kg b wt (100 mg tablets all of the same brand and batch Tibinide® F

Table I *Estimates of kinetic parameters of INH in nine healthy male volunteers given a single oral dose on an empty stomach and together with a standardized breakfast*

t_{max}=estimated time of peak concentration k_1=influx (absorption) constant k_2=elimination constant $t_{\frac{1}{2}}$=elimination half life AUC=serum concentration curve area

Subj no	Observed absorption delay (min)	t_{max} (min)	k_1	Absorption half life (min)	Observed peak con centration (μg/ml)	k_2	$t_{\frac{1}{2}}$ (min)	Empirical AUC ∫360	Mean con centra tion
Fasting									
1	15	90	0 01191	58	7 1	0 01464	47	1 206	3 50
2	10	30	0 14991	5	12 6	0 008942	78	1 251	3 57
3	5	30	0 12276	6	14 0	0 007720	89	1 376	3 89
4	50	80	0 09112	8	8 8	0 007392	94	1 242	4 01
5	20	90	0 02603	27	8 5	0 006752	103	1 307	3 84
6	10	45	0 07773	9	8 6	0 006404	108	1 560	4 46
7	30	50	0 21740	3	12 7	0 002985	232	2 735	8 79
8	40	90	0 05899	12	12 3	0 003721	186	2 314	7 23
9	5	15	0 00352	2	45 8	0 003523	197	4 070	11 50
Non fasting									
1	10	90	0 01673	41	4 4	0 009054	76	823	2 33
2	35	110	0 02044	34	3 8	0 008110	85	710	2 19
3	25	90	0 02668	26	3 4	0 007841	88	698	2 08
4	25	110	0 02109	33	3 9	0 005704	122	823	2 46
5	40	120	0 01819	38	3 1	0 008139	85	575	1 80
6	60	150	0 01940	36	2 5	0 005605	124	538	1 79
7	10	100	0 02675	41	3 4	0 003257	215	1 873	5 35
8	40	105	0 04693	15	5 9	0 002636	263	1 365	4 27
9	15	115	0 02358	29	8 9	0 003016	230	2 295	6 65
Satistical significance of difference (paired observations) between fasting and non fasting conditions			N S		$p<0\ 001$	N S	N S	$p<0\ 001$	$p<0\ 001$

Malmo Sweden)—was ingested either together with 100 ml of drinking water or immediately after a standard ized breakfast The breakfast prepared by a dietician was composed of 150 ml low fat milk 100 ml orange juice 1 egg 2 pieces of crisp bread 5 g margarine 20 g orange marmalade and 20 g cheese This equaled 20 g (20%) protein 17 g (35%) fat and 50 g (45%) carbohydrates and a total energy of 1 840 kJ (440 kcal) About 100 ml non sweetened black coffee was included The dietician or the nurse collecting the blood samples sur veilled eating and intake of tablets When the tablets were taken on an empty stomach the subjects abstained from food and liquid for another two hours after drug adminis tration

Blood samples (about 10 ml) were obtained before (0 hour) and at about 5 10 15 30 45 60 75 90 105 120 150 180 240 300 and 360 min after drug ingestion The exact time (adjusted to the nearest minute) of blood sampling (when the sampling tube was half filled) was re corded and used in calculations and graphs Before each blood sampling 1–2 ml blood was obtained and discarded and after each sampling 2–4 ml 0 15 M saline was injected via the cannula The blood samples were left at room temperature for more than one but less than two hours They were then centrifuged and serum was collected and frozen at −20°C till the next morning when the measure ment of INH concentrations was carried out The INH assays were always performed on the day after the ex periment since it had been discovered that the amount of INH in a frozen standard sample changed with time A spectrophotometric technique (5) was used to assess the levels of unmetabolized INH in serum

The absorption and elimination of the drug were as sumed to follow first order kinetics Ideally the formula for the blood concentration of INH (*y*) in relation to time (*t*) would then be

$$y=a\,\frac{k_1}{(k_2-k_1)}\,(e^{-k_1t}-e^{k_2t})$$

where k_1 is the influx (absorption) constant k_2 the elimina tion constant and *a* a factor proportional to the dose (7) The constant k_2 was estimated from the linear part of the semilog plot (log *y* vs time) Together with t_{max} the time of peak concentration in blood k_2 was used to assess k_1 by iterative approximation on a computer to assure a four digit estimate The delay in onset of full influx (absorp tion) was judged by eye from the graphs The trapezoidal integral of the experimental curve ∫exp from the initial point of full influx until 360 min was determined on a computer The integral of the blood concentration curve ∫calc between the initial point of full influx and 360 min

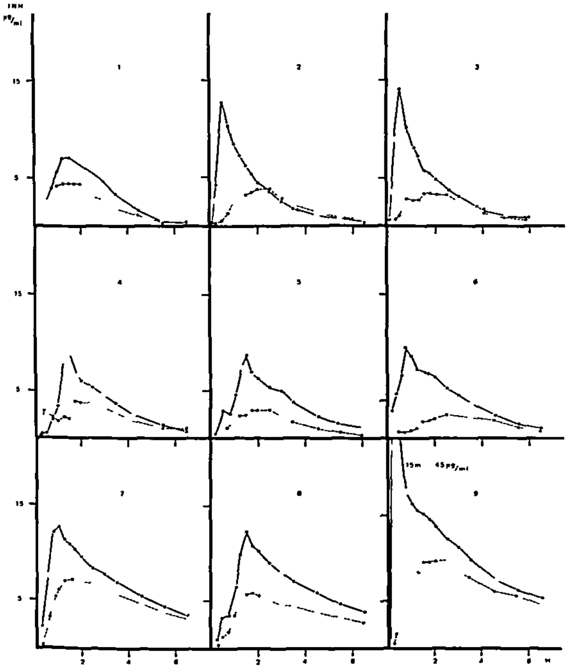


Fig 1 Serum levels (μg/ml) of unmetabolized isoniazid (INH) in nine healthy male volunteers after a single oral dose (100 mg/10 kg) given on a fasting stomach (—) and together with a standardized breakfast ( ) In each subject both the peak concentration values and the areas under the serum concentration curves were greatly reduced The differences between fasting and non-fasting values (paired observations) were highly significant

was calculated for a=1 Using a=fexp/fcalc the curve was calculated and superimposed on the experimental graph Dividing fexp by (360 – initial point of full influx) yielded the mean serum level during the experiment

Statistical differences were assessed by the *t* or the *F* test on paired observations

RESULTS

It is generally agreed that the rate of INH acetylation is genetically determined and that subjects can be classified as rapid or slow acetylators by estimation of the half life of INH elimination from serum According to this criterion there were three slow ($t_{1/2}$>3 hours) and six rapid ($t_{1/2}$<2 hours) acetylators (Table I Fig 1) Some of the latter may in fact be intermediates From semilog plotted curves it appeared that under fasting conditions there were both slow and rapid acetylators whose INH levels seemed to reflect a rapid distribution phase preceding a slower elimination phase In most subjects however the elimination phase seemed to completely overlap the distribution phase Fig 1 and Table I show also that there were great interindividual differences in both the peak concentrations and the values of the areas under the serum concentration curves (AUC) during fasting as well as non fasting conditions

Whether he was a rapid or a slow acetylator each subject had lower mean serum levels of INH when the drug had been ingested after the breakfast than when it had been taken on an empty stomach Indeed both the peak concentrations and the AUC values were reduced in each individual (Fig 1 Table I) and both the difference between fasting and non fasting mean peak values mean AUC values and mean concentration values were highly significant (Table I) In eight of the nine subjects the absorption rate was decreased when the drug was taken in the postprandial state but this difference did not reach statistical significance (Table I)

In the postprandial curves fewer recordings could be assumed with certainty to belong to the elimination phase thus rendering the estimation of the elimination rate less accurate During non fasting conditions the calculated elimination half life was slightly prolonged in some but slightly reduced in some subjects The mean values (fasting vs non fasting) were not significantly different (Fig. 1 Table I)

DISCUSSION

A major reason for the clinical assessment of blood concentrations of drugs is the assumption that the blood level is related to the effective drug concentration at the site of drug action and hence to the therapeutic effect For INH it has been observed that, when the drug is given in single daily doses its therapeutic effect is best associated with the peak serum concentration whereas its chronic toxicity appears to be related to the total amount absorbed (6)

The present study indicates that concomitant food intake strongly reduces the bioavailability of INH as both the peak concentration the mean concentration and the total amount absorbed were diminished INH is assumed to be absorbed primarily from the intestine and not from the stomach (1 4) and administration of antacids together with INH has been found to reduce its absorption probably as a consequence of delayed gastric emptying (3) Delay in gastric emptying consequent to food intake could hence be a likely explanation of the present findings, but interactions of INH and various food components may also be important Irrespective of the mechanism involved the observations motivate a strictly defined dosage regimen for INH in the treatment of tuberculosis with the drug always given on an empty stomach Moreover the decision about dose size should be based on assessments of single-dose kinetics of INH When only information on the rate of acetylation is needed fasting conditions are not mandatory but preferable

Indeed the great interindividual differences in INH levels recorded even within the rapid and slow acetylator subgroups suggest that the acetylation rate as such may be an insufficient parameter for the proper adjustment of the dosage regimen for INH hydralazine and other drugs subject to the same mode of biotransformation

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Lactate Production during Fructose Infusion with or without Amino Acids

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ABSTRACT Lactate production from the liver during fructose infusion was decreased when an amino acid infusion was given simultaneously The most pronounced decrease was observed when the amino acid infusion was started before the simultaneous administration of fructose and amino acids The explanation of the phenomenon is thought to be a stimulation of gluconeogenesis by amino acids

It is known that fructose given intravenously is metabolized more rapidly than equivalent amounts of glucose (9) This has been attributed to higher activity of ketokinase in the liver in comparison with glucokinase and unspecific hexokinase (20) Fructose taken up by the liver is partly metabolized to lactate which is released into the general circulation (15) If the rate of infusion of fructose is high metabolic acidosis may ensue which is potentially dangerous to patients with pre-existing acidosis (1 5)

It has been shown that i v amino acid solutions are better utilized if carbohydrate is also given simultaneously and it has been suggested that fructose is superior to glucose in favoring amino acid utilization (11 12) Since fructose as well as amino acid solutions are nowadays used for parenteral nutrition it would be of interest to know whether amino acids infused simultaneously with fructose exert any effect on the metabolism of fructose In the experiments reported here standardized infusions of fructose or/and amino acid solution were given either alone or simultaneously and the effects on the concentrations of various metabolites in peripheral blood were studied In addition a few experiments with liver vein catheterization were performed in order to further elucidate the effects of amino acids on fructose metabolism

Our results indicate that formation of lactate from fructose in the liver is strongly inhibited by i v amino acid administration

MATERIAL AND METHODS

Young healthy male and female volunteers were studied They had no history of metabolic disorder and routine chemical analyses performed on blood samples prior to the investigation were normal The subjects were not on a controlled diet They had not performed any physical work for at least 12 hours before the experiments The studies were started between 8 and 12 a m after an overnight fast

Infusions of fructose and amino acid solutions were given through indwelling venous catheters using constant infusion pumps When the solutions were infused simultaneously they were given in different peripheral veins Fructose was given as a 20% solution at an infusion rate of 1 g/kg b wt /hour The amino acid solution (Intramin forte Astra Sodertalje) contained eight essential and a number of non-essential amino acids The composition is presented in Table I

The infusion rate was 50 mg amino acid nitrogen/kg b wt /hour Peripheral venous blood was collected before infusion and at intervals during infusion for determination of glucose fructose lactate pyruvate β-hydroxybutyrate and acetoacetate

Hepatic vein catheterization for studies of splanchnic metabolism was carried out as follows Under X ray control a catheter was positioned through vena femoralis with the tip in a central hepatic vein Catheters were also inserted in a peripheral artery and in two peripheral veins A constant infusion of indocyanine green was given through one of the venous catheters Blood samples from the hepatic vein and the peripheral artery were taken at

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Table I Amino acid solution

	g/l	g N/l
L phenylalanine	7 70	0 65
L-isoleucine	4 90	0 52
L leucine	7 70	0 82
L lysine	5 60*	1 07
L methionine	7 70	0 72
L threonine	3 50	0 41
L tryptophan	1 80	0 25
L valine	5 60	0 67
L histidine	6 00*	1 63
L arginine	10 00*	3 22
L alanine	16 00	2 52
Glycine	10 00	1 87
L proline	10 00	1 22
L serine	5 00	0 67
L aspartic acid	8 70	0 92
pH 6 5–7 0		
Total	110 20	17 16

* As acetate

taneously at intervals during the experiments The splanchnick blood flow was determined according to the Fick principle as described by Bradley et al (6) and by *Castenfors et al* (7)

All the experiments were preceded by 3 basal periods lasting about 10 min each before infusion of fructose or amino acids Lactate pyruvate β hydroxybutyrate and acetoacetate were determined in whole blood by enzymatic methods (2 13) and glucose by the orthotoluidine method described by Hultman (14)

Simultaneous determination of glucose and fructose in blood was performed using a modification of the orthotoluidine and MAP (11) methods described by Bergstrom and Hultman (4) Determinations were made in plasma of urea (8) and indocyanine green (16) The respective uptake and production of metabolites in the splanchnic area were calculated by multiplying the arterial hepatic–venous differences and the estimated splanchnic blood flow

The following types of infusion were studied A) Fructose infusion during 4 hours 5 subjects B) Infusion of fructose and amino acids simultaneously 4 subjects of whom 1 had hepatic vein catheterization C) Fructose infusion during 90 min followed by simultaneous infusion of fructose and amino acids 5 subjects of whom 2 had hepatic vein catheterization D) Infusion of amino acids during 90 min followed by simultaneous infusion of fructose and amino acids 5 subjects of whom 2 had hepatic vein catheterization

RESULTS

A *Fructose infusion without amino acids* An increase in the blood glucose concentration was observed during the first 90 min of infusion without amino acids (Table II A) The blood fructose con-

Table II *Venous blood concentrations of glucose fructose lactate and pyruvate (mmol/l) before and during fructose and amino acid infusions*

	Time after start of infusion (min)	Glucose		Fructose		Lactate	
		n	*x*±S E M	*n*	*x*±S E M	*n*	*x*±S E M
Basal values		22	5 30±0 16			22	0 55±0 012
A Fructose infusion without amino acids	30	9	5 98±0 32	9	3 32±0 25	9	2 22±0 26
	60	10	5 97±0 29	10	4 05±0 36	10	3.54±0 16
	90	6	6 20±0 47	6	3 96±0 36	5	4 01±0 29
	150	5	5 17±0 29	5	5 28±0 37	5	3 15±0 19
	240	5	5 27±0 26	5	5 53±0 42	5	2 96±0 25
B Simultaneous infusion of fructose and amino acids	30	4	6 04±0 25	4	3 51±0 34	4	2 38±0 09
	60	4	4 81±0 35	4	4 00±0 27	4	2 34±0 36
	90	4	4 08±0 24	4	4 40±0 07	4	2 14±0 76
	120	4	4 35±0 56	4	4 15±0 14	4	2 20±0 74
C Fructose infusion for 60–90 followed by simultaneous infusion of fructose and amino acids	30 ª	5	5 79±0 48	5	4 22±0 51	5	2 91±0 31
	60 ª	5	4 94±0 49	5	4.31±0 26	5	2 71±0 79
	90 ª	5	5 26±0 45	5	4.38±0 22	5	1 97±0 13
D Amino acid infusion for 60–90 followed by simultaneous infusion of fructose and amino acids	30 ᵇ	5	6 29±0 46			5	0 52±0 047
	60 ᵇ	5	5 99±0 47			5	0 64±0 004
	90 ᵇ	2	6 48			2	0 60
	30 ª	5	6 07±0 56	5	2 89±0 28	5	1 16±0 068
	60 ª	5	5 53±0 43	5	3 77±0 38	5	1 44±0 146
	90 ª	5	5 68±0 39	5	4 22±0 29	5	1.37±0 14

ª Time after start of fructose and amino acid infusion ᵇ Time after start of amino acid infusion

on increased gradually during the infusion value being recorded after 240 min The lactate concentration increased markedly from 55 to a maximum of 4 01 mmol/l after 90 min decreasing again to 2 96 mmol/l The concentration also rose The relative in was however smaller As a consequence the lactate/pyruvate ratio increased gradually up to 4 after 240 min of fructose infusion (Table II A)

B *Simultaneous infusion of fructose and amino acids* The infusion lasted for 120 min except for the subjects with hepatic vein catheterization for whom infusion time was 240 min The glucose concen after 60–120 min of fructose and amino acid infusion were significantly lower than at corresponding times during infusion of fructose alone

The blood fructose concentration did not differ between the two groups during the first 90 min of infusion The lactate concentration was much lower after 60–120 min of fructose and amino acid infusion than after 60–150 min of fructose infusion without amino acids In these subjects the pyruvate concentration was not determined (Table II B) In the experiment with hepatic vein catheterization we

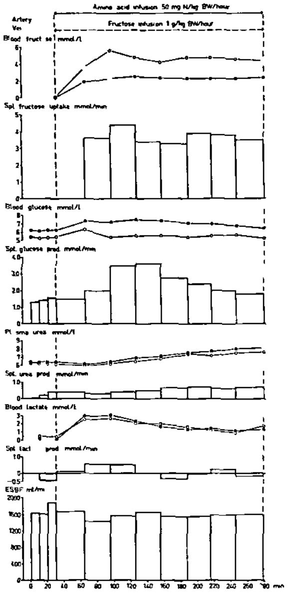


Fig 1 Estimated splanchnic blood flow (*ESBF*) and splanchnic metabolism in a normal subject before and during infusion of fructose and amino acids for 245 min ●–●–●=arterial blood ○–○–○=liver vein blood

found only a slight lactate production at the beginning of the infusion During the second half we even found a slight splanchnic uptake of lactate (Fig 1) This contrasts markedly with the steep rise of splanchnic lactate production observed earlier in liver vein catheterization experiments in which fructose was given without amino acids (3 4) and in the two experiments of the present study in which fructose was first given alone for 90 min

vate	Lactate/pyruvate	
$x \pm$S E M	n	$x \pm$S E M
0 069±0 0050	16	8 13±0 875
0 200±0 015	9	11 4 ±0 55
0 241±0 023	10	15 2 ±0 75
0 308±0 051	4	14 0 ±1 36
0 168±0 014	5	18 9 ±0 73
0 113±0 022	5	28 4 ±3 25
0 228±0 035	5	13 3 ±1 36
0 17?±0 028	5	16 4 ±1 23
0 167±0 0061	5	12 5 ±1 15
0 094±0 015	5	5 98±1 03
0 090±0 008	5	7 38±1 45
0 114	2	5 31
0 1?4±0 014	5	9 76±1 14
0 132±0 0?0	5	12 4 ±2 73
0 131±0 023	5	13 0 ±3 91

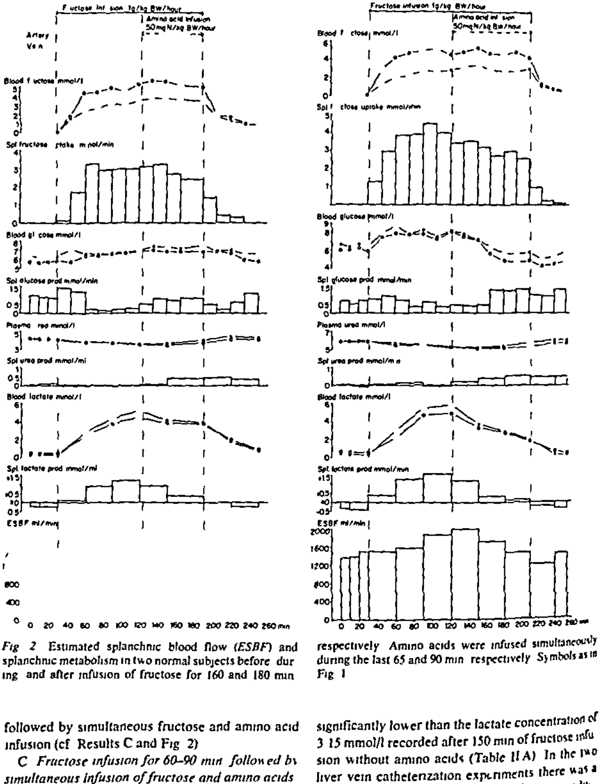


Fig 2 Estimated splanchnic blood flow (*ESBF*) and splanchnic metabolism in two normal subjects before during and after infusion of fructose for 160 and 180 min respectively Amino acids were infused simultaneously during the last 65 and 90 min respectively Symbols as in Fig 1

followed by simultaneous fructose and amino acid infusion (cf Results C and Fig 2)

C *Fructose infusion for 60–90 min followed by simultaneous infusion of fructose and amino acids* After amino acid infusion had been started the lactate concentration fell reaching a minimum of 1 97 mmol/l after 90 min of simultaneous fructose and amino acid infusion i e 150–180 min after the start of the fructose infusion (Table II C) This is significantly lower than the lactate concentration of 3 15 mmol/l recorded after 150 min of fructose infusion without amino acids (Table II A) In the two liver vein catheterization experiments there was a very marked increase in splanchnic lactate production during the 90 min when fructose was given alone As soon as the amino acid infusion was added the production of lactate decreased rapidly and a splanchnic uptake was observed immediately af

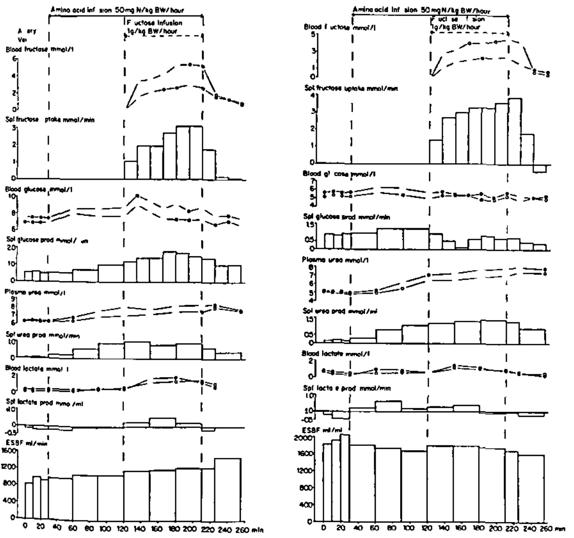


Fig 3 Estimated splanchnic blood flow (*ESBF*) and splanchnic metabolism in two normal subjects before during and after infusion of amino acids for 180 min Fructose was infused simultaneously during the last 90 min Symbols as in Fig 1

ter the end of the infusion in spite of a continued splanchnic uptake of fructose (Fig 2) During fructose infusion alone there was also a decrease in splanchnic glucose production confirming earlier results (3 4) but when amino acids were added to the infusion glucose production again rose to the initial level (Fig 2) As with the infusion of fructose alone there was an increase in pyruvate and a marked increase in the lactate/pyruvate ratio as well (Table II C)

D *Amino acid infusion for 60–90 min followed by simultaneous infusion of fructose and amino acids* An increase in blood glucose concentration was recorded when the amino acids were infused alone The level remained essentially unchanged during the subsequent simultaneous infusion of fructose and amino acids The lactate concentration was not significantly changed during the amino acid infusion When the infusion of fructose was added blood lactate rose slightly reaching a maximum after 60 min of simultaneous infusion (Table II D) The lactate concentrations after the infusion of fructose had been added to that of amino acids were all significantly lower than when fructose and amino acid infusions were started simultaneously (Table II B) or when fructose infusion was started before

amino acid infusion (Table II C) This indicates a more effective inhibition of lactate production from fructose by amino acids when the amino acid administration precedes the simultaneous infusion In the two hepatic vein catheterization experiments we observed a rise in splanchnic urea production during the amino acid infusion (Fig 3) Amino acid infusion alone did not influence the splanchnic lactate metabolism After the start of fructose infusion there was only a slight and transient increase in lactate production (Fig 3) The lactate/pyruvate ratio during this part of the infusion increased significantly as in all other groups during fructose infusion (Table II D)

E *Acetoacetate and β-hydroxybutyrate* The basal values for acetoacetate were 0 03 *mmol/l±0 007 (S E M) and for γ hydroxybutyrate* 0 153±0 020 (n=23) During all periods with fructose infusion and also during amino acid infusions without fructose the concentrations of acetoacetate and γ hydroxybutyrate decreased to values less than 0 02 and 0 05 mmol/l respectively indicating that fructose as well as the amino acid mixture had a pronounced antiketogenic effect

DISCUSSION

The most significant finding in the present study is the lower level of lactate in blood when fructose was infused simultaneously with the amino acid solution than when fructose was infused alone The hepatic vein catheterization experiments revealed that the effect was due to an inhibition of the splanchnic production of lactate which presumably takes place in the liver The lowest level of lactate in blood and also the most efficient inhibition of splanchnic lactate formation from fructose was found when the amino acid infusion was started some time before the beginning of the fructose administration This indicates that a supply of amino acids needs to be present for some time before fructose is given in order to exert its full inhibiting effect on the formation of lactate from fructose in the liver

Furthermore in the experiments in which fructose infusion was started before or at the same time as the amino acid infusion the amino acids gradually exerted an inhibiting effect on splanchnic lactate production The strong inhibition of lactate formation from fructose in the liver brought about by the amino acids administered indicates that the latter exert a profound influence on the metabolism of fructose The fact that the uptake of fructose by the liver was practically unchanged by the amino acid infusion indicates that the first step in fructose metabolism i e the phosphorylation of fructose is not inhibited For the same reason inhibition of the aldolase reaction which is one of the rate limiting steps is also probably not inhibited Further along the path to pyruvate and lactate pyruvate kinase is thought to be a rate limiting step This enzyme is inhibited by alanine and phenylalanine (19) both of which were included in the amino acid infusion However alanine infusion together with fructose did not inhibit lactate production (Bergström et al unpublished observations) Another explanation could be that amino acid infusion stimulates the *activity of gluconeogenic enzymes thus increasing* the capacity of the gluconeogenic pathway up to glucose and glycogen

The decrease in glucose production from the liver during fructose infusion was observed earlier (4) The restoration of glucose production to preinfusion level when amino acids are added speaks in favor of the theory that gluconeogenesis is stimulated The pronounced effect of the amino acids on the hepatic production of lactate from fructose is of clinical interest since it indicates that fructose can be given with less danger of acidosis from excessive lactate formation provided amino acids are included in the infusate

ACKNOWLEDGEMENTS

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The Tromsø Heart Study

Methods and Main Results of the Cross sectional Study

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ABSTRACT The mortality from coronary heart disease (CHD) in Norway increased rapidly during 1951–70 the highest mortality rates as well as the most rapid increases being found in Northern Nor way Several surveys of CHD were then planned one of them is reported here All men 20–49 years of age, living in the municipality of Tromsø, Troms county, were called up for examination In total, 6 595 men 74 4% of those invited, were examined Cholesterol, triglyceride and Hb values, BP, body weight and height, the percentage of smokers and cigarette consumption have been tabulated according to area age, work schedule health condition, physical activity and ethnic background The results suggest that the relatively high mortality from CHD in Northern Norway is associated with high serum cholesterol concentrations as well as a relatively high prevalence of smoking During the screening there were indications of changes in dietary habits in the municipality, presumably as a result of ac companying publicity

The mortality ascribed to coronary heart disease (CHD) increased rapidly in Norway during 1951–70 (Table I) The age and cause specific mortality on county level was analysed for the first time for the period 1959–62 Unexpectedly the northernmost county Finnmark had the highest CHD mortality rate even higher than Oslo (7) Certification pe culiarities or a higher case fatality could not ex plain the findings which contrasted with the previous conception of CHD as primarily an urban disease (20) Finnmark being the most sparsely populated county in Norway Further analysis of the 1959–62 material for the two northernmost counties Troms and Finnmark revealed that the mortality rate is at least as high in the thinly populated areas as in the small urban areas of the region

The next county mortality analysis was per formed for the period 1964–67 The increase in the CHD mortality rate since 1959–62 in the three northernmost counties was clearly above the natio nal average for both sexes (8)

A similar analysis for the period 1969–72 shows that the increase in the mortality rate from CHD in northern Norway as in other parts of the country has declined There is no further increase in the gap between the mortality in the three northern coun ties and the national mortality (9) The data for the age group 40–69 age adjusted from the three afore mentioned county analyses are summarized in Table II In addition to Oslo and the three counties of Northern Norway data are given for a low risk county on the west coast Sogn og Fjordane Note that even for a 4 year period the number of deaths is so small that the rates have a considerable random error

The essence of epidemiology is to study areas with high and low incidences and areas where the situation changes significantly When the 1964–67 county analysis became available the University of Tromsø decided to undertake a population study of CHD The Tromsø study to be reported here is one among several related studies the Oslo City Health Department in cooperation with Oslo Municipal Hospital has completed a large CHD survey of males in Oslo while the State Mass Radiography Service is carrying out screening studies of males and females in Finnmark county (high risk) and Sogn og Fjordane (low risk) The studies have been made as comparable as possible examination of serum cholesterol BP and smoking

Table I *Mortality from coronary heart disease in Norway in 1951–72 per 1 million per year*

Age group	1951–55	1956–60	1961–65	1966–68	1969–70	1971–72
Men						
30–34	33	43	82	54	98	70
35–39	98	115	228	193	210	255
40–44	214	376	530	652	693	642
45–49	581	866	1 046	1 407	1 401	1 451
Women						
30–34	8	12	10	3	10	19
35–39	16	14	31	32	25	47
40–44	33	40	54	64	68	85
45–49	74	122	131	116	166	165

habits forming a common base (21 22 34 35 36) Attempts will be made to correlate these factors to certain social and environmental variables In the years to come the cause specific mortality will be analysed for all the persons called up for examination and hospitalized cases of myocardial infarction will be registered as completely as possible

MATERIAL AND METHODS

The municipality of Tromsø is located at a latitude of approximately 70°N (400 km north of the Arctic Circle) and covers 2 432 km² (Fig 1)

The population studied included all men in the municipality born in 1925–54 being 20–49 years of age at the time of the study The registration was based on the official census of Sept 1 1973 The total number of men registered was 8 867 of whom 935 lived outside the community at the time of the invitation to the examination.

The number of men examined was 6 593 of whom 46? responded after receiving a second letter The response rate was 83 1 % of those able to participate and 74 4 % of the registered population The response rates for different age groups and areas are given in Table III

The men were examined at the Out patient Clinic of the University Hospital approximately 10 days after receiving by mail questionnaire I and a request to participate in the study In areas III and IV 273 fishermen were examined at the fishing stations mostly in the afternoons and evenings a deviation from the usual procedure that turned out not to influence the results to any notable degree

Table II *Mortality from coronary heart disease among Norwegian men aged 40–69 in 1959–72*

County	1959–62	1964–67	1969–72
Mortality in 1959–62=100			
Oslo	100	102	108
Sogn og Fjordane	100	121	150
Nordland	100	129	137
Troms	100	143	140
Finnmark	100	124	116
All counties	100	119	126
Mortality in Norway=100			
Oslo	135	115	115
Sogn og Fjordane	61	62	73
Nordland	102	110	111
Troms	102	123	114
Finnmark	145	151	134
All counties*	100	100	100

* Standardized rates per 100 000 population 1959–62 333 1964–67 396 1969–72 418

Questionnaires and examination

Questionnaire I comprised six main topics A) Previously known atherosclerotic disease hypertension or diabetes mellitus B) Symptoms possibly caused by coronary or peripheral atherosclerosis (a translation of the questionnaire given by Rose (31) was used for this purpose) C) Physical activity during leisure D) Smoking habits E) Conditions of work–physical activity etc F) Ethnic origin G) Family history of CHD Questionnaire II which was presented at the examination gave room for detailed family histories of CHD a material to be reported in another publication

In general the subjects were examined between 8 a m and 4 p m No instructions had been given to change dietary or other habits The examination lasted for 8–10 min and was organized as follows 1) Placing of sphygmomanometer cuff 2) Completion and/or correction of questionnaire I 3) First BP measurement 4) Completion of questionnaire II 5) Second BP measurement 6) Collection of venous blood sample 7) Weight and height measurement Five specially trained secretaries and two physicians (D S T & O H F) participated in the examinations

Blood pressure

The BP was read to the nearest even number of mmHg and measured at 4–5-min intervals with a mercury sphyg-

Table III *Population size and number of subjects examined according to age and area*

Area I urban centre II=farmers and commuters III fishermen commuters farmers IV fishermen fishing industry
N population *n* no. of men examined

Age group	Area I		Area II		Area III		Area IV		Total		
	N	*n*	*N*	*n*	*N*	*n*	*N*	*n*	*N*	*n*	Percent age of *N*
20–29	2 912	1 875	321	210	216	152	208	150	3 657	2 387	65.3
30–39	2 460	1 919	217	156	169	144	145	125	2 991	2 344	78.4
40–49	1 798	1 506	162	133	135	118	124	107	2 219	1 864	84.0
Total	7 170	5 300	700	499	520	414	477	382	8 867	6 595	74.4

momanometer on the left upper arm with the subject in a sitting position. The systolic BP was measured when the first Korotkoff sound appeared (phase 1) and the lowest reading was recorded. The diastolic BP was defined as the pressure at the disappearance phase of the Korotkoff sound (phase 5). If there was no phase 5 the pressure at phase 4 was recorded.

The secretaries and physicians had been trained according to a program used in the Oslo study based on tape recorded BP measurements produced by the London School of Hygiene and Tropical Medicine (26). The monthly averages of the BPs measured by each secretary and physician were used as a control of the technique (2, 12). The differences between the averages were satisfactory (10 mmHg) for all months except April (13.1 mmHg). Control computations indicated that the lower BP erroneously recorded in that month did not notably influence the results.

Weight and height were measured to the nearest kg and cm, the men wearing shirt and trousers but not shoes.

Venous blood sample

Blood samples for triglyceride and cholesterol determinations were collected from a cubital vein using Vacutainer cat. no. 4710. The analyses were performed at the Division of Clinical Chemistry, Institute of Medical Biology.

Hemoglobin determination and standardization

The Hb determination and standardization were performed according to the recommendations of the International Committee for Standardization in Hematology (19).

Cholesterol determination

Total cholesterol concentration in serum was determined manually by a Liebermann Burchard procedure using the reagent described by Huang et al. (18). In a cuvette 1.0 ml of reagent was added to 0.025 ml of serum and immediately blended on a Vortex mixer. The absorbance was read after 15 min at room temperature at 617 nm on an LKB 7400 photometer. The readings were performed against 0.025 ml of water in 1.0 ml of reagent.

Cholesterol standardization

A standard solution of human β and pre β lipoproteins in 0.9% saline was prepared after heparin $MnCl_2$ precipitation (4) and kept frozen at −20°C in small batches. The cholesterol concentration in the lipoprotein solution was determined by using Cholesterol CH S Sigma Chemical Company (200 mg/dl in acetic acid) as a primary standard in the following manner. To the cuvettes containing the lipoprotein solution and the acetic standard were added 0.025 ml of acetic acid and 0.025 ml of water respectively. Mean values of 10 determinations were used for estimating the cholesterol concentration of the lipoprotein solution. A secondary standard was used as the velocity of the development of the Liebermann-Burchard colour is highly dependent on the water content of the

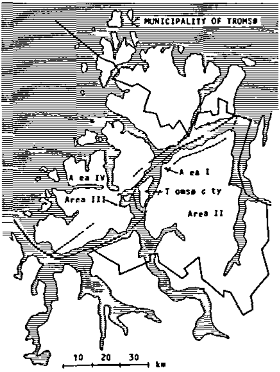


Fig. 1. The municipality of Tromsø with subdivision into areas used in the present paper.

Table IV *Cholesterol and triglyceride analyses of control serum Liponorm Nyco batch 50 during the screening period*

Recommended values cholesterol 249 mg/100 ml triglycerides 1 86 mmol/l

	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
Cholesterol (mg/100 ml)												
No of analyses	25	65	54	44	54	111	103	128	141	158	44	977
Mean	271	265	257	263	259	262	265	262	258	262	265	262
S D	6 6	10 2	12 1	4 5	4 6	3 1	5 0	5 3	3 5	4 2	3 0	6 4
Triglycerides (mmol/l)												
No of analyses	30	37	40	38	36	52	68	75	75	67	35	553
Mean	2 07	2 04	1 89	1 93	1 89	1 81	1 79	1 83	1 85	1 85	1 82	1 8[illegible]
S D	0 12	0 09	0 11	0 11	0 06	0 05	0 04	0 04	0 04	0 05	0 04	0 0[illegible]

reaction mixture The number of serum samples in each series of analyses was kept below 50 At least three determinations of the standard solution were performed for each series and the mean value of the absorbance readings was used for calculating the serum concentrations

Triglyceride determination and standardization

Triglyceride concentration was determined manually by the method of Soloni (33) slightly modified (15) A solution of Tripalmitin (T 8002 Sigma Chemical Corp St Louis Miss USA) in the extraction solvent was used as a standard for the procedure The absorbance of the lutidine derivative was read at 409 nm on the LKB 7400 photometer The reagent blank consisted of water instead of serum carried through the whole procedure Mean values of three determinations of the standards were used to calculate serum concentrations

Quality control of the cholesterol and triglyceride determinations

Liponorm Nyco batch no 50 was used for the daily control of the triglyceride and cholesterol determinations Within each series of analyses 2–4 Liponorm samples were analyzed The mean values of the Liponorm for each month of the screening period are given in Table IV The mean Liponorm cholesterol value obtained at our laboratory was 262 mg/100 ml which is 13 mg/100 ml above the recommended value of the manufacturer (249 mg/100 ml) However a value of 260 mg/100 ml was obtained by the manufacturer using an AutoAnalyzer procedure

To further test the accuracy of the method 74 consecutive human serum samples accompanied by 5 Liponorm control samples were also analyzed by an enzymatic method specific for 3 β hydroxysteroids (31) The mean value of the human serum samples was 240 mg/100 ml using the enzymatic method and 245 mg/100 ml using our ordinary method Approximately half of the difference can be explained by postulating a mean bilirubin concentration of 0 5 mg/100 ml in the human samples leaving 2–3 mg/100 ml to be explained by unspecific chromogens influencing the direct Liebermann-Buchard assay The bilirubin concentration of the Liponorm control samples was found at our laboratory to be 1 3 mg/100 ml leading to a supposed systematic error in the cholesterol determinations of the control samples of 5 mg/100 ml Analyzed with the enzymatic method the Liponorm control samples gave cholesterol values very close to the recommended value Therefore the Liponorm serum seems to contain unspecific chromogens leading to a systematic error in the cholesterol determinations of the control samples of about 8 mg/100 ml

The total mean of the triglyceride analyses of the Liponorm control serum was 1 87 mmol/l close to the recommended value of 1 86 mmol/l

The first four monthly results of the Liponorm analyses showed certain variations which are difficult to explain (Table IV) Some of the means and S D were clearly above the corresponding values found in the following months However the variations in laboratory technique thus revealed may be regarded as tolerable

All things considered the accuracy of our cholesterol and triglyceride determinations seems to have been satisfactory

Data processing

A punchcard file identifying the individuals by the 11 digit national identification number names and addresses was obtained from the municipal Population Register The file was used for making sets of adhesive labels and pre identified punchcards as well as for constructing direct access magnetic files After having been collected the information was key punched and read into the main magnetic file while improbable values were listed for control The data were also controlled by proofreading the entire main file against the original forms Patients to be referred for intervention were listed automatically and individual reports were printed Using a general cross tabulation program control statistics were produced at regular intervals (24)

Statistical methods

Age adjustment for subgroups was obtained by calculating an expected mean according to the indirect method

Table V *Serum lipids blood pressure hemoglobin concentration body weight and height (mean values according to age) percentage of smokers and their mean daily consumption of cigarettes in Group C (healthy men)*

Age group	n^a	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)	BP (mmHg) Systolic	BP (mmHg) Diastolic	Hb (g/100 ml)	Weight (kg)	Height (cm)	Smokers (%)	Cigarette consumption
20–24	938	215	1 21	124 2	73 4	14 92	71 5	178 0	56 7	13 5
25–29	1 306	239	1 37	125 7	75 9	14 93	74 3	177 6	59 0	13 9
30–34	1 253	253	1 41	125 1	76 8	14 81	75 5	177 5	52 5	15 6
35–39	945	269	1 52	126 8	79 0	14 83	76 4	176 6	53 7	15 4
40–44	828	278	1 48	127 9	80 5	14 74	76 6	176 1	56 3	15 0
45–49	794	285	1 57	129 4	82 0	14 72	75 9	175 3	59 2	14 9
Total	6 064	254	1 42	126 3	77 6	14 84	75 0	177 0	56 1	14 7

[a] No of subjects included in the estimation of mean serum cholesterol concentration For the other averages the number of subjects is identical or slightly lower

Statistical significance was determined by using the "large sample t test" t being calculated using the formula

$$t=\frac{|x_1-x_2|}{\sqrt{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}}$$

(x_1 and x_2 being age adjusted mean values s_1 and s_2 the S D of the observed values)

Grouping of the subjects

The subjects were classified according to the answers in questionnaires I and II into groups according to

Health condition A) Affirmative answers to questions concerning past or present atherosclerotic disease hypertension or diabetes mellitus B) Affirmative answers to the questions formulated by Rose (31) concerning exercise induced chest pain and/or calf pain of short duration C) Subjects classified neither as A nor B (healthy subjects)

Physical activity 1) Athletes (competitive sportsmen training hard several times a week) 2) Heavy workers 3) Men of sedentary habits 4) Others not classified as 1 2 or 3 (ordinary moderate activity)

Ethnic origin 1) Lappish (two or more grandparents of Lappish origin) 2) Finnish (two or more grandparents of Finnish origin) 3) Uncertain (presumably in most cases Norsemen of partly Lappish origin) 4) Subjects not classified as 1 2 or 3 (mainly of Norse origin)

Geographic areas (Fig 1) I) The urban centre of the municipality of Tromsø and its nearest surroundings II) Surrounding fjords inhabited mostly by farmers and commuters working in area I III) Inner coastal region inhabited by fishermen commuters farmers IV) Outer coastal region inhabited by fishermen and workers in the fishing industry

Work schedule 1) Shift and night workers 2) Commuters (men not returning home from work every day)

Family history of CHD Men reporting one or more first-degree relatives with a history of myocardial infarction or angina pectoris

RESULTS

Single Variables and Their Relation to Age

The relations of serum cholesterol triglyceride BP weight height smoking habits and cigarette consumption to age are illustrated in Table V which is based on group C (healthy subjects only)

Figs 2 and 3 show the relation of cholesterol triglycerides and systolic and diastolic BP to age The age dependent change per year was estimated from straight regression lines computed for each ten year interval from unweighed one year averages

Serum cholesterol concentration

The mean serum cholesterol value was 215 mg/100 ml for age group 20–24 and 285 mg/100 ml for age group 45–49 The yearly increase with age was 4 85 mg/100 ml for ages 20–29 2 96 mg/100 ml for ages 30–39 and 1 15 mg/100 ml for ages 40–49 The frequency distribution of the serum cholesterol concentrations for all men examined is shown in Fig 4

Serum triglyceride concentration

In this unfasting population the mean serum triglyceride value was 1 21 mmol/l for age group 20–24

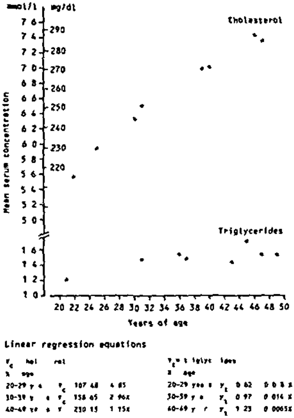


Fig 2 Increase in serum cholesterol and triglyceride concentrations with age Single year mean values group C (healthy men)

and 1 57 mmol/l for age group 45–49 The mean serum triglyceride value increased by 0 028 mmol/l/year for ages 20–29 For age group 30–39 the increase was 0 014 mmol/l/year After the age of 40 there was no notable increase in the serum triglyceride concentration The frequency distribution of serum triglyceride concentrations for all men examined is shown in Fig 5

Systolic and diastolic blood pressure

The total mean systolic BP was 126 3 and the total mean diastolic BP 77 6 mmHg The increase with age appears to be roughly linear

Hemoglobin concentration

The total mean Hb concentration was 14 84 g/100 ml There was a slight decrease with age the mean being 14 92 g/100 ml for age group 20–24 and 14 72 g/100 ml for age group 45–49

Height and weight

The mean height was 176 89 cm decreasing with age from 178 0 cm in age group 20–24 to 175 3 cm in age group 45–49 The mean weight was 75 1 kg increasing with age from 71 4 kg for age group 20–24 to 76 3 kg for age group 45–49

Cigarette smoking

The prevalence of smoking was highest among the youngest and oldest subjects examined The percentage of current daily smokers was 59% among the subjects in age group 20–29 compared with 5… in age group 30–39 while 57% were daily smokers in age group 40–49 The mean daily cigarette consumption of smokers was 14 7 cigarettes It differed only slightly between age groups the lowest consumption being found in the youngest subjects As neither the percentage of daily smokers nor the cigarette consumption seemed to rise or fall with age in a regular way the tabulated values concerning smoking habits have not been age adjusted

Single Variables in the Whole Material and Subgroups

Table VI shows age adjusted mean values of single variables in different subgroups

Health condition

Group A those with known diseases related to CHD comprised 220 men Compared with group…

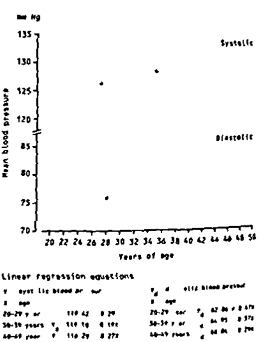


Fig 3 Increase in systolic and diastolic blood pressure with age Single year mean values group C (healthy men)

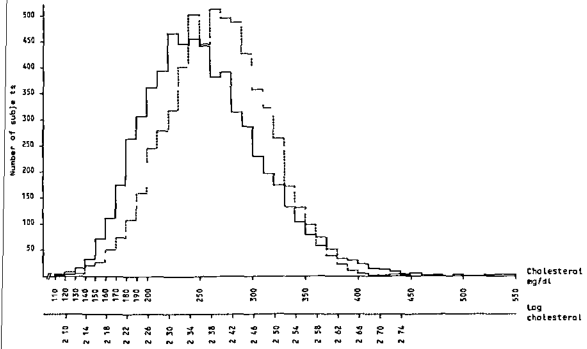


Fig 4 Serum cholesterol and log serum cholesterol con centrations Frequency distributions all men examined

group A had a higher mean age higher mean serum cholesterol (p<0 05) and serum triglyceride values (p<0 001) and higher systolic and diastolic BP (p<0 001) The frequency of smoking how ever was lower

Group B those giving an affirmative answer about symptoms implying coronary or peripheral atherosclerotic disease comprised 309 men They showed higher mean serum cholesterol values (p<0 05) heavier daily cigarette consumption (p<0 01) and a lower body height (p<0 01) than the men in group C

Physical activity

The athletes as a group had the lowest mean serum cholesterol value significantly lower than the other subjects taken together (p<0 001) In contrast subjects with physically heavy work had the highest mean serum cholesterol value higher than the other subjects taken together (p<0 05) The athletes had the lowest mean diastolic BP significantly lower than the other subjects taken together (p<0 01) Subjects with physically heavy work had the highest mean systolic BP higher than the other subjects taken together (p<0 001) Thus the two groups reporting the highest levels of physical activity are contrasts with respect to cholesterol values and BP

Athletes and heavy workers were also contrasts with respect to smoking as 32 7% of the athletes reported daily smoking against 69 6% of the heavy workers Also the daily cigarette consumption of the smoking athletes was low 11 6 cigarettes per day while that of the heavy workers was near to the average for the total population namely 14 8 cigarettes a day

Ethnic origin

Men reporting Finnish or Lappish origin had higher mean serum cholesterol values than the subjects of Norse origin (p<0 001 in both) Men of uncertain ethnic origin occupied an intermediate position Men of Lappish origin had a significantly lower diastolic BP (p<0 01) and a lower body height than those of Norse origin Of the ethnic groups men of Lappish origin had the highest percentage of daily smokers

Work schedule

Men reporting commuting or night and shift work had a higher mean serum cholesterol concentra tion than men with other work schedule (p<0 01)

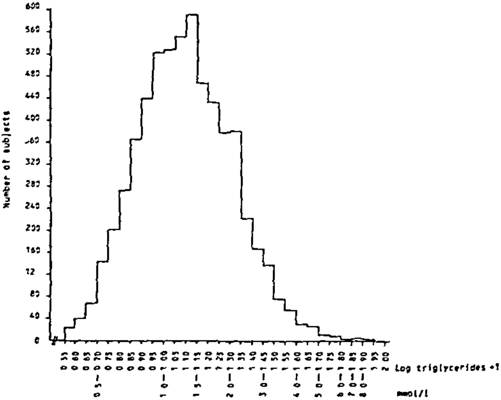


Fig 5 Serum triglyceride concentration given as the log+1 Frequency distributions all men examined

The frequency of smokers and their daily cigarette consumption were also higher among the former categories of workers

Family history of CHD

... reporting CHD among their first-degree relatives had a higher mean serum cholesterol concentration than those with no such family history ($p<0.01$)

Geographical areas and seasonal variations

Men from the rural and coastal areas II III and IV had higher mean serum cholesterol concentrations and higher mean systolic and diastolic BP than men living in the urban area I ($p<0.001$) a difference which cannot be explained by seasonal variation alone The mean serum triglyceride concentration was also higher among subjects from the rural and coastal areas ($p<0.01$) Smoking was most frequent in areas II and III

Monthly age adjusted mean values for different variables in area I are shown in Table VII The subjects examined in Feb and Dec were recruited selectively as the examinations in Feb took place outside the hospital in the evenings and aft... noons while the subjects examined in Dec w... the responders to a second call up letter

The monthly mean serum cholesterol va... showed an almost linear decrease from March ... July amounting to 29 mg/100 ml (Table VII) a... thereafter an increase of 9 mg/100 ml from July ... Aug , but subsequently no notable increase

The monthly mean systolic and diastolic ... showed a decrease during the spring months ... lowest mean values being registered in July a... Aug respectively There was a subsequent ... crease in the autumn to the level of early spr... giving a total seasonal variation for systolic BP ... 6 2 mmHg and for diastolic BP of 8 7 mmHg

DISCUSSION

A main problem brought forward by the CH... mortality pattern in the three northernmost counti... of Norway is whether the mortality can be ... plained by the known predisposing factors or ... is necessary to postulate an influence of specifi... unknown factors The results of this study sugg...

Table VI *Serum lipids blood pressure hemoglobin concentration body weight and height (age adjusted mean values for all men examined and for certain defined groups) percentage of smokers and their mean consumption of cigarettes (not age adjusted)*

Group	n	Cholesterol (mg/100 ml)	Triglycerides (mmol/l)	BP (mmHg) Systolic	BP (mmHg) Diastolic	Hb (g/100 ml)	Weight (kg)	Height (cm)	Smokers (%)	Cigarette consumption
All men examined										
	6 593	255	1 43	126 5	77 9	14 84	75 1	176 89	56 8	14 7
Grouping according to health condition										
A	220	266	1 64	132 7	82 9	14 93	76 9	177 15	51 8	13 8
B	309	263	1 47	125 1	77 5	14 86	75 9	175 73	58 2	16 2
C	6 064	255	1 42	126 3	77 7	14 84	75 0	176 94	56 1	14 7
Grouping according to physical activity (group C only)										
Athletes	233	235	1 34	125 3	74 4	14 60	74 2	176 45	32 7	11 6
Heavy workers	704	266	1 45	129 6	79 3	14 87	75 5	176 07	69 6	14 8
Sedentary life	690	256	1 48	125 5	78 4	14 94	76 2	177 03	62 7	16 5
Others	4 437	253	1 41	125 9	77 3	14 84	74 8	177 10	55 0	14 5
Grouping according to ethnic background										
Finnish	308	269	1 44	126 6	79 0	14 86	74 8	174 78	58 1	14 6
Lappish	152	276	1 55	127 1	76 4	14 77	69 9	171 18	63 8	15 5
Uncertain	925	261	1 44	126 8	78 8	14 79	73 9	174 98	60 9	14 5
Others	5 208	253	1 43	126 4	78 2	14 84	75 5	177 52	55 7	14 8
Grouping according to area										
I	5 298	251	1 40	125 6	76 4	14 80	75 2	177 40	55 6	14 9
II	499	261	1 31	128 7	78 0	14 62	72 5	172 82	64 8	13 5
III	414	282	1 72	129 2	82 5	15 22	75 7	176 41	62 3	14 3
IV	382	283	1 72	133 5	83 9	15 16	76 6	175 76	57 5	14 3
Grouping according to work schedule										
Shift workers	1 291	262	1 48	127 2	79 1	14 96	76 6	176 69	64 9	15 7
Day workers	5 254	254	1 41	126 3	77 5	14 80	74 7	176 93	54 8	14 4
Commuters	1 172	266	1 44	127 7	78 8	14 93	76 3	176 07	66 6	16 6
Others	5 367	253	1 42	126 2	77 6	14 81	74 8	177 06	54 6	14 2
Grouping according to CHD among first degree relatives										
Yes	1 762	260	1 44	127 1	78 5	14 84	75 0	176 62	58 6	15 2
No	4 719	254	1 41	126 3	77 6	14 83	75 1	177 00	55 9	14 6

No of subjects included in the estimation of mean serum cholesterol concentration For the other averages the number of subjects is identical or slightly lower Subjects who could not be grouped because of missing information were excluded

that the high mortality from CHD in Northern Norway is associated with high serum cholesterol concentrations and relatively high consumption of cigarettes Regarding the third known risk factor blood pressure the registered values were well in accordance with findings made elsewhere in Norway (5)

The role of the minority populations of Lapps and Finns in influencing the risk level of CHD in Northern Norway is sometimes speculated on Men of Lappish and Finnish descent in the present population are found to have a slightly increased risk but they are too few to appreciably influence the total mortality

The mean cholesterol level in the population investigated is among the highest reported The mean of 285 mg/100 ml for Tromsø males aged 45–49 may be compared with values for males aged

Table VII *Serum lipids blood pressure hemoglobin concentration body weight and height according to month of screening (age adjusted mean values for men from area I)*

	Feb	March	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
*n**	27	129	199	863	261	214	1 034	880	881	594	216	5 [illegible]
Cholesterol (mg/100 ml)	283	270	262	255	242	241	250	251	249	251	244	251
Triglycerides (mmol/l)	1 76	1 66	1 67	1 57	1 42	1 43	1 35	1 33	1 33	1 28	1 [illegible]	1 [illegible]
BP (mmHg)												
Systolic	133 1	127 0	126 0	125 0	124 6	121 6	123 3	126 5	127 1	127 0	126 0	125.6
Diastolic	82 6	82 6	80 5	79 1	77 5	74 5	73 9	76 6	76 9	79 1	77 0	64
Weight (kg)	76 6	75 8	75 5	75 4	74 5	74 7	74 6	74 8	75 8	75.5	74 6	75 2
Height (cm)	178 6	177 0	177 8	177 7	177 8	177 2	177 3	177 4	177 0	177 0	177 6	177 4
Hb (g/100 ml)	15 20	15 15	15 30	14 92	14 81	14 71	14 67	14 78	14 80	14 78	14 59	14 [illegible]

* No of subjects included in the estimation of mean serum cholesterol concentration For the other averages the number of subjects is identical or slightly lower

40–49 in other areas in Norway (29) 298 7 mg/100 ml for infarction patients in Oslo 270 2 mg/100 ml for employees in Oslo 257 6 mg/100 ml for fishermen on the West coast and 222 8 mg/100 ml for farmers in a mountainous area in the South However still higher serum cholesterol values have been reported in a small population in Finnmark county in Northern Norway (13)

The high cholesterol levels presently reported cannot be explained by the fact that the subjects were not fasting (17) The postprandial rise of blood lipids is due to an increase of both the very low density lipoproteins and the chylomicrons Most studies on fasting subjects show a mean triglyceride concentration of 1 0 mmol/l (14) The difference between this and the present mean of 1 4 mmol/l may be ascribed to the postprandial effect on the lipid concentration If the postprandial effect revealed by this difference were caused by very low density lipoproteins the total serum cholesterol concentration would increase by approximately 7 mg/100 ml if it were caused by chylomicrons the increase would be only about 0 5 mg/100 ml Therefore the postprandial effect on the cholesterol concentration in this study appears to have been below 5 mg/100 ml which is hardly of consequence for the conclusions

Comparing cholesterol values obtained in different studies is difficult because of the different methods used but a comparison of age trends may be more informative In the present material yearly increases with age were 4 85 and 2 96 mg/100 ml for men aged 20–29 and 30–39 respectively For comparison data regarding telegraph employees in Oslo (29) when analysed by unweighed regression on the basis of mean values gave yearly increases of 4 76 and 4 56 mg/ml respectively for these age groups The increase in cholesterol values with age found in the latter material was one of the highest reported at that time (1962) For the whole age span of 20–49 years the present material showed a yearly increase of 2 79 mg/100 ml which may be compared with the yearly increase of 2 29 mg/100 ml in Minnesota men aged 17–45 (23) In our material the yearly increase in the third and fourth decades of life is markedly greater than in the fifth decade The study of Minnesota men as well as a large cooperative study performed in the US (25) reported a straighter linear association between age and serum cholesterol The present age trend may indicate that the Tromsø men in general attain a relatively high serum cholesterol level at an early age The "levelling off" in the third and fourth decades may be interpreted in two ways The finding may reflect the true development of the cohorts or it may mean that the younger cohorts in general have higher cholesterol values than the older

The monthly mean serum cholesterol concentrations found in the present study clearly demonstrate seasonal variation the lowest mean value being found in July However the values did not increase in the autumn to the levels found in April and May During the screening period the grocers in the area reported increasing sales of skimmed milk soft margarine and vegetable oils presumably as a result of publicity associated with the screening This change in dietary habits may be the reason why the monthly mean serum cholesterol did not show the expected increase during the autumn months which may indicate that it is

possible to lower the serum cholesterol level in a population and thus lower the risk for CHD

The percentage of daily smokers was 56 8 in the present study while data published by the Central Bureau of Statistics in 1973 indicated that 43% of Norwegian men aged 25–45 were daily smokers (11) However the cigarette consumption of the smokers in the present study was only a little above the national average Although comparisons are complicated by differences in methods there is hardly doubt that the male population in Tromsø because of smoking habits is running a higher risk for acquiring CHD than Norwegian men in general

The third main risk factor BP largely exhibits a general level as well as an age related increase in accordance with values reported previously e g our values are almost identical to those found by the Bergen Blood Pressure Study (5)

The average triglyceride concentrations found here are higher than those reported elsewere but the non fasting conditions under which the blood samples were drawn hinder direct comparisons

Some of the subgroups of the population may be defined as high risk groups based on the three major risk factors serum cholesterol BP and smoking These subgroups are 1) commuters 2) shift workers 3) heavy workers 4) subjects living in geographical areas III and IV 5) subjects of Finnish and Lappish descent A contrast is formed by the groups designated as heavy workers and as athletes Both groups represent a high level of physical performance but the heavy workers were found to be a high risk group and the athletes a low risk group Also persons living in the rural part of the community seem to be at higher risk than anticipated

The high risk found by us for heavy workers contrasts with the findings by Medalie et al (27) and Morris et al (28) of a higher incidence of CHD for men with sedentary habits Other studies however have not confirmed the supposed association between high physical activity and declined CHD mortality (1 30)

The contrasts between heavy workers and athletes as well as between persons living in the rural parts of the municipality and those living in the center may be partly explained by social status Thus the rural part of the municipality may not be inhabited by a rural population in the traditional sense but by a population shifting to an urban way of life with the men occupied mainly as workers

Most probably the high overall level of serum cholesterol reflects dietary habits in the population On the other hand higher serum cholesterol levels have been observed in populations in the winter than in the summer (3 6 11) It is possible that the special climatic conditions in this area with low mean temperatures long winters and short cold summers may influence the serum cholesterol level

The primary conclusions of the present study are that the elevation of the risk factors serum cholesterol blood pressure and smoking seems to be associated with residential area type of occupation and to some extent ethnic origin It remains to be elucidated how these conditions operate in determining the level of risk There is probably a relation between the high level of risk factors and the high incidence of CHD in the area Other factors such as the familial occurrence of CHD may also contribute to the incidence of CHD The predictive power of these factors and their interrelationships however cannot be estimated until the prospective part of the study has been completed

Further study of the Tromsø population will concentrate on the following topics 1) The relation between the main risk factors and other social and environmental factors 2) The relation between the main risk factors and the incidence of CHD 3) The relation between the incidence of CHD among the examined subjects and among their first degree relatives 4) Intervention procedures for men classified as having a high risk of CHD

ACKNOWLEDGEMENTS

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Project committee A Nordøy chairman A Forsdahl A Hertzberg P Kraft J H Strømme K Westlund and the authors

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Three-year Follow-up of Middle-aged Men with Low Blood Pressure

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ABSTRACT The blood pressure (BP) development in 142 middle aged men with BP within the lower region of the BP distribution (supine BP≤120/70 mmHg) has been studied Over a 3 year period there was a moderate but significant rise in supine systolic BP but not in supine diastolic BP However, no subject developed hypertension defined as supine BP≥175/105 mmHg These findings support the assumption that among middle aged men the hypertensives are recruited from the upper part of the BP distribution Subjects with low BP may be rescreened at longer intervals if at all

There is an increased risk of developing hypertension in subjects with borderline blood pressure (BBP) (1 6 7 8 11 12) The risk has been estimated to be 2–4 times that of normotensives Several studies have shown that there is an increase in systolic blood pressure (SBP) as well as diastolic blood pressure (DBP) with age (2 3 4 13) It has also been found that persons with *higher* initial readings show a steeper BP rise (9)

In a previous study of middle aged men with BBP the incidence of hypertension was 26% over a 3 year period (6) The aim of the present investigation was to evaluate the BP development in subjects of the same population study with the lowest BPs

MATERIAL

Between Sept 1970 and Sept 1973 a health examination was offered all men living in the City of Uppsala and born in 1920–24 (5) The participation rate was 83 9% The study was planned as a feasibility trial aiming to change risk factors for cardiovascular disease

The present study comprised men born in 1922 From this age group 459 men (86 4%) participated in the health examination All men with a supine BP≤120/70 mmHg (n=162) were after 3 years invited to a reexamination *in which 142 men (87 7%) participated*

METHODS

The initial examination as well as the reexamination were performed under similar conditions The subjects were asked to be fasting and to have refrained from smoking since the preceding midnight The examination started at 7 30 a m and the BP was measured by the same registered nurse on both occasions Each individual had his two examinations in the same season of the year

The BP was measured on the right arm after lying prone for 10 min and after 2 min in sitting position Mercury manometers (Kifa Ercameter wall model) were used The BP cuff had a rubber bladder 12 5 cm wide and 35 cm long SBP and DBP were read to the nearest 5 mmHg mark The DBP was measured at the disappearance of the Korotkoff sounds (phase 5)

Conventional statistical methods were used for calculation of mean value and S D Significance of differences between mean values was estimated with Student s t test (2 tailed test) Regression analyses were performed by the method of least squares The accepted level of significance was $p<0.05$

RESULTS

The average BPs of the 142 subjects at screening and after 3 years are shown in Table I With the exception of supine DBP there was a significant rise in BP over the 3 year period None of the subjects had received antihypertensive therapy between the two examinations

Twenty seven men had received dietary treatment for hyperlipidaemia and another five for impaired glucose tolerance The average supine BP of these 32 men at screening was 119 8/72 3 mmHg

Table I *Mean systolic (SBP) and diastolic (DBP) blood pressure (mmHg) in 142 men at screening and after 3 years*

	Screening examination		After 3 years		
	Mean	S D	Mean	S D	*p* value
SBP					
Supine	117 0	8 7	120 7	11 5	<0 005
Sitting	119 2	10 7	122 8	13 2	<0 02
DBP					
Supine	74 4	7 6	76 0	7 6	>0 05
Sitting	80 9	7 0	82 9	7 7	<0 05

The corresponding value for the remaining 110 men was 116 2/75 0 mmHg The SBP of these 32 men was significantly higher than that of the remaining group ($p<0\ 05$) and after 3 years the BP was 121 4/73 3 mmHg not significantly higher than at the screening

The changes in supine BP are shown in Fig 1 Sixty three men (44 4%) had higher SBP than 3 years earlier and 62 (43 7%) had higher DBP Both pressures had increased in 39 subjects (27 5%) On the other hand 31 men (21 8%) had lower SBP than 3 years earlier and 41 (28 9%) had lower DBP Both pressures had decreased in 11 subjects (7 7%)

The initial SBPs and DBPs in relation to the values after 3 years are shown in Figs 2 and 3 respectively There was a significant correlation between the SBPs as well as between the DBPs The highest SBP at the screening was 170 mmHg but this pressure had not increased over the 3 year period No subject had a SBP≥170 mmHg at the *second examination Only 6 individuals had SBP≥* 150 mmHg The highest DBP at the screening was 95 mmHg which was recorded in one subject After 3 years only 2 subjects had this pressure and none had a higher DBP

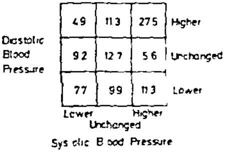


Fig 1 Changes (%) *in supine blood pressure over a 3 year period in 142 men with initial BP≤120/70 mmHg*

The average initial body weight of the total material of 142 subjects and of the untreated 110 individuals was the same 74 3±10 6 kg The values after 3 years were 0 2 and 0 9 kg higher respectively The average body weight of the 32 men in the intervention group was 74 6±10 6 kg *at screening and 2 3 kg lower at the second examination* The difference was not significant

No significant differences were found when the BP development over the 3 year period was analysed in relation to body weight weight gain or initial pulse rate

DISCUSSION

We found in a previous study from the same health examination survey (6) that among 93 men with borderline raised BP 14 subjects developed hypertension (supine BP≥175/105 mmHg) and another 11 men received antihypertensive therapy over a 4-year period This motivates repeated BP meas-

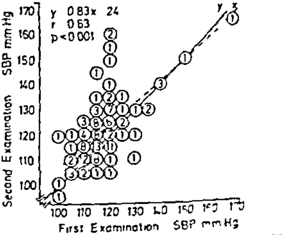


Fig 2 Relation between supine systolic blood pressure (SBP) in 142 men initially and after 3 years

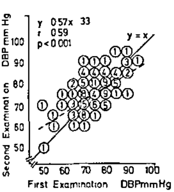


Fig 3 Relation between supine diastolic blood pressures (*DBP*) in 142 men initially and after 3 years

urements However it would be of value if some people could be left without further examination In the present investigation the BP development was studied in individuals with BP within the lower region of the BP distribution

No subject developed hypertension—defined as supine BP≥175/105 mmHg—over the 3 year period However there was a significant rise in supine SBP This rise was about half that of the subjects with BBP (6) This is in agreement with earlier findings that subjects with higher initial readings showed a steeper BP rise (9) It also ought to be pointed out that when BPs at one end of the distribution are measured a second time the statistical phenomenon of regression towards the mean must be taken into account (10)

If one assumes that the BP development was the same among those with supine BP≤120/70 mmHg who did not come to the second examination the study has shown that none of 35 3% of the population with the lowest BPs became hypertensive over the 3 year period It is also unlikely that the initial participation rate (86 4%) in this age group could influence this assumption Therefore the present findings may be compared with those of the earlier study from the same population survey (6) where 25 5% of the subjects with BBP were defined as hypertensives after an equal length of time

These results support the assumption that among middle aged men the hypertensives are recruited from the upper part of the BP distribution This also means that subjects with low BP can be rescreened at longer intervals if rescreening is deemed to be indicated at all

ACKNOWLEDGEMENT

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Complication Rates of Selective Percutaneous Transfemoral Coronary Arteriography

A Review of 1 094 Consecutive Examinations

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ABSTRACT A review is presented of 1 094 selective coronary artery studies during a 3-year period in which 7 001 coronary artery injections were performed using the percutaneous transfemoral artery approach as described by Judkins A total of 24 serious complications occurred, including 11 ventricular fibrillations, 5 asystoles or severe bradycardias 3 acute myocardial infarctions and 5 cerebral vascular accidents There were 5 deaths, giving an over all mortality rate of 0 46 % Causes of individual complications are analyzed The incidence of serious cardiac complications did not differ significantly from that reported in the literature with the Sones technique The Judkins technique is a simple and reliable method for selective coronary arteriography In our opinion the incidence of complications can be kept at an acceptably low level by meticulous examination techniques

Coronary arteriography has become one of the most important diagnostic procedures in modern cardiology providing information about the anatomy and structural changes of the coronary arteries in living man The first technique to be introduced that of Sones (14) is performed from a brachial arteriotomy More recently the percutaneous transfemoral technique was described by Judkins (7) This method employs preshaped catheters introduced percutaneously into a femoral artery and advanced over a guide wire to the aortic root Guide wires are not used with the transbrachial approach Both methods represent an inherent risk Recently several reports have described a higher incidence of complications with the use of the Judkins technique compared with the Sones technique (2 4 11 15) Information about the incidence of complications with either of these two methods is needed in particular because of the current interest in coronary artery surgery since the magnitude of the risk for mortality and morbidity should be reckoned as part of the total complications

The percutaneous technique for coronary angiography via the transfemoral route has been used exclusively in our laboratory since 1968 The purpose of the present communication is to present our data for the morbidity and mortality in serious cardiac complications with this method during a 3 year period

MATERIAL AND METHODS

During the 3 year period 1972–74 covered by the present investigation 7 001 selective coronary arteriographies were performed on a total of 1 094 patients varying in age from 20 to 65 years The most common indication for the investigation was the assessment of patients with coronary heart disease Coronary arteriography was also performed in some patients before valve replacement and with the diagnosis of other forms of heart disease such as cardiomyopathy non-cardiac chest pain congenital anomalies of the coronary arteries and cardiac failure before and after valve replacement The number of patients examined and the number of arteriograms performed each year are presented in Table I

The Judkins technique was used in all patients Separate catheters were used for the right and left coronary arteries The left coronary artery catheter is advanced first A teflon-coated safety guide wire is used to facilitate entry of this catheter When the injections in the left coronary artery have been completed the left coronary artery catheter is exchanged for the right coronary catheter over a guide wire The right coronary

Table I *Patients examined and selective arteriograms performed annually and annual number of complications relative to the number of patients examined and number of arteriograms performed*

	1972	1973	1974	Total
No of pats	*278*	*373*	*443*	*1 094*
No of arteriograms	1 779	2 387	2 835	7 001
No of complications	11	8	5	24
in % of pats examined	3 96	2 14	1 13	2 20
in % of arteriograms performed	0 62	0 34	0 18	0 34

catheter is advanced to just above the bifurcation of the aorta and the guide wire is removed Free backflow of blood should occur through this catheter before it is ad vanced any further The catheter is attached through a three way stopcock to a pressure drip of normal saline containing 5 U heparin/mm The pressure at the catheter tip is monitored continuously No injection is made if the catheter tip pressure is unusually damped The total duration from femoral artery puncture to end of coronary injections was 30–45 min in most cases

The patients were examined in a postabsorptive state Premedication including atropine was given routinely The contrast medium used was Isopaque Coronar® containing originally bound iodine in a solution of sodium meglumine and calcium salt of metrizoic acid (Table II) Hand injections of 5–8 ml contrast medium were given repeatedly

The complications we considered to be of major im portance were 1) Ventricular fibrillation 2) Bradycardia including severe sinus bradycardia (<40 beats/min) second degree or complete heart block 3) Acute myocardial infarction with ECG evidence of myocardial necrosis and later confirmed by serum enzyme analysis 4) Cerebral vascular accident

Detailed records of all complications have been kept during the period 1972–74 with a view to such an ana lysis as this and helped to clarify the precise nature of the complications Standard ECG leads I and II were re corded during the investigation Autopsy reports for all patients who died within 24 hours after the catheteriza tion were reviewed and analyzed All available coronary arteriograms were reviewed personally by the authors

Table II *Essential data on Isopaque Coronar® 370 mg J/ml*

Content of meglumine metrizoate (mg/ml)	656 5
Additional ionic content (mEq/l)	
Na	155
Ca	17
Proportional content of meglumine/Na/Ca metrizoate salts	6 6 1 0 1
Viscosity (cP)	
20°C	17 3
37°C	8 5
Osmolality (37°C) (mol/kg)	2 15

RESULTS

A total of 24 major complications were registered giving an incidence of 24/1 094 (2 20%) for all patients studied and 24/7 001 (0 34%) for all arteriograms performed A total of 21 complications were directly related to the catheterization of one of the coronary arteries while changing of the guide wire in the aorta was the precipitating cause in 3 patients The incidence of complications among the annual numbers of cases and arteriograms performed is given in percentages in Table I The incidence varied but tended to decrease in spite of a growing number of examinations The incidence of complications related to left coronary artery catheterization was 8/3 839 procedures (0 21%) while the incidence of complications related to catheterization of the right coronary artery was 13/3 062 (0 42%) Five patients died, giving an over all mortality rate of 5/1 094 (0 46%) or 5/7 001 (0 07%) of all examinations The fatalities related predominantly to catheterization of the left coronary artery (Table III) A fresh thrombus was found at autopsy in one patient who had normal coronary arteries In the other deceased patients no thrombus was found but it is noteworthy that this series had occlusive disease in two major branches of the coronary arteries

Ventricular fibrillation (VF) was the most common complication occurring in 11/1 094 patients (1 01%) Seven episodes of VF occurred after right coronary catheterization or 7/3 062 procedures (0 23%) and 4 episodes after left coronary artery catheterization or 4/3 939 procedures (0 10%) VF was reversible by DC countershock in all but 2 patients who died

Asystole complete heart block or severe sinus bradycardia occurred in 5/1 094 patients (0 46%) These episodes followed catheterization of the right coronary artery in all but one patient Three cases reverted to sinus rhythm Abnormally high serum levels of digitoxin probably contributed to the fatal outcome in one of the two patients who died

Acute myocardial infarction (AMI) occurred in 3/1 094 patients (0 27%) one of whom died No thrombus was found at autopsy

Cerebral vascular accident was observed in 5/1 094 patients (0 46%) presenting in most cases with focal localizing signs and more unusual with generalized cerebral disturbances In 3 patients

Table III *Classification of deaths with autopsy findings*

Ventricular fibrillation
(57 year old male)
Total occlusion in left anterior descending and right coronary artery Diffuse atherosclerotic narrowing in circumflex artery Old myocardial infarction No thrombus

Ventricular fibrillation
(62 year-old male)
Coronary arteries open without atherosclerotic narrowing Fresh thrombus in circumflex artery with recent AMI

Asystole
(67 year-old female)
Total occlusion in left anterior descending and right coronary artery Diffuse atherosclerotic narrowing in circumflex artery No thrombus

Asystole
(58-year-old male)
Total occlusion in left anterior descending and right coronary artery Moderately atherosclerotic changes in circumflex artery No thrombus

Acute myocardial infarction
(67 year-old female)
Subtotal occlusion in left anterior descending and circumflex artery Total occlusion in right coronary artery AMI No fresh thrombus

symptoms occurred immediately after the catheter had been advanced over a guide wire to the aortic root In 2 patients symptoms followed catheterization of the left coronary artery All patients made an uneventful recovery

DISCUSSION

In the early years of coronary arteriography the risk of fatal complications was reported to be as low as 0 1-0 3% *These data were mainly based on results* obtained by the transbrachial approach and from one centre (14) More recent reports have shown that the risk of fatalities had risen 5–6 times following the introduction of the transfemoral route (2 4 11 15)

The risk of complications reported by us is based on data obtained at one centre and with one technique the transfemoral or Judkins technique The 0 46% mortality rate from coronary arteriography is lower than usually reported from centres using the transfemoral technique and is identical with or only slightly higher than the rate of mortality reported from centres using the transbrachial technique and doing the same volume of cases (Table IV) It is noteworthy that a fresh thrombus was present at autopsy in only one of our autopsy cases The other patients who died had advanced 2–3 vessel disease with no fresh thrombus

In the present study as in other similar series *VF was the most common complication (1 01%)* In studies by McIntosh (9) VF occurred in 23/3251 patients (0 8%) Adams and Fraser (2) found VF or prolonged arrhythmias in 1 15% (brachial approach) and 1 41% (femoral approach) Bank et al (3) using both methods had 5 episodes of VF in 809 arteriograms yielding an incidence of 0 8% which is higher than the overall incidence of 11/7001 (0 16%) found by us (based on the number of arteriograms performed) Marked bradycardia or asystole was a less common complication (0 46%) Digitalis intoxication was considered to be the precipitating cause in one patient McIntosh (9) reported serious bradycardia or asystole in 4 (0 05%) of 7062 patients studied while Bank et al (3) had two episodes of bradycardia/asystole in 2 (0 3%) of 809 arteriograms performed In accordance with other investigators VF or bradycardia/asystole was much more easily provoked by arteriographic examination of the right than the left coronary artery (5) The predominance of rhythmic disturbances associated with catheterization of the right coronary artery probably reflects the fact that the AV node in man is primarily supplied by the right coronary artery Interference with this region of the conduction system might either depress conduction leading to heart block or asystole or induce a second burst of activity causing various dysrhythmias ending with VF Theoretically left coronary artery injection may cause bradycardia due to stimulation of chemoreceptors limited to *the left ventricle and induce reflex changes in the* heart characterized by both bradycardia and hypo-

Table IV *Influence of technique on mortality*

	Transbrachial		Transfemoral	
	No of pats	Incidence	No of pats	Incidence
Kaltenbach & Lichtlen (8)	1 367	0 4	431	0 9
Chahine et al (4)	413	0 0	478	2 0
Petch et al (11)	111	0 0	248	1 5
Adams & Fraser (2)	24 124	0 13	22 780	0 78
Present study			1 094	0 46

tension (6) Serious tachy/bradyarrhythmias may also be due to the contrast medium itself (13) The composition of the contrast agent used in the heart is critical and the range of acceptable ion concentrations in general and the sodium content in particular is small A concentration of sodium close to the physiological level as in Isopaque Coronar® (155 mEq/l) is in accordance with recommended figures (10)

Coronary arteriography was associated with the development of AMI in 3 patients (0 27%) and caused cerebral embolic episodes in 5 (0 46%) Both conditions are important because they may be due to embolization from a thrombus of the catheter tip or dislodged atheromatous material from the inside of the arterial wall Myocardial infarction may also be related to prolonged catheter obstruction of a stenotic lesion dissection of a coronary vessel or possible spasm secondary to catheter manipulation The incidence of AMI in association with coronary angiography is reported to be higher with the Judkins technique than with the Sones technique (2 4 16) However in a recent extensive survey Adams and Adams (1) found that the incidence of AMI was 0 23% irrespective of the method applied Data on the incidence of cerebral emboli are not easily derived from the literature Adams and Fraser (2) found a minimum incidence of 0 23% with a significantly higher occurrence using the transfemoral than the brachial approach 0 43% vs 0 03% In a more recent report (1) these figures are modified to 0 09% and 0 10% respectively

Studies of the literature thus show that the two common methods of coronary arteriography brachial selective and femoral selective both carry an inherent risk As far as technique is concerned we believe that the transfemoral route is probably as safe as the transbrachial Coronary arteriography is easier and quicker to perform by this technique which also avoids the problem of brachial arterial occlusion It is also apparent from the present study as well as from a few other recent studies in this field that the incidence of death serious arrhythmias AMI and cerebral embolism is not necessarily higher when utilizing the transfemoral approach (1, 12) The serious complications primarily thromboembolic (17) may be reduced if antithrombogenic material is used to coat guide wires and catheters or more successfully together with single dose total body heparinization (1) Since thrombus formation may be related to platelets adhering to the catheter salicylic acid may prevent the thrombotic complications

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Attempted Diagnosis of Ventricular Mural Thrombi in Acute Myocardial Infarction Using ^{125}I-labelled Fibrinogen

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ABSTRACT In an attempt to diagnose ventricular mural thrombi complicating acute myocardial infarction (AMI), 80 patients have been given 100 μCi ^{125}I labelled fibrinogen after admission to a CCU Precordial radioactivity was recorded for the following 6 days over four sites corresponding to chest leads CR_1–CR_4 A sustained rise in radioactivity of at least 15% of initial recordings was classed as type A pattern, a minor rise or flattened response as type B pattern and a rapid decrease as type C pattern 28% showed a type A 19% a type B and 54% a type C pattern There was no significant difference between the groups in incidence of pericardial friction rub but when patients with suspected pericarditis (as evidenced by characteristic pains) were added, pericarditis was significantly overrepresented in the type A group Smaller infarctions (SGOT<100 U/l) were significantly more common in patients with a type C decay pattern No differences were noted between the groups as regards type and site of the infarction A sustained rise in precordial radioactivity after an AMI may be an indication of mural thrombosis but the influence of other factors secondary to an infarction e g pericarditis, cannot be determined at present

Formation of mural thrombi in the ventricular cavities after acute myocardial infarction (AMI) poses a clinical problem because of the potential risk of embolization No method which is feasible to perform routinely exists for the diagnosis of ventricular mural thrombi in vivo A simple method for the detection of mural thrombi would therefore be of great value for an understanding of the natural history of this complication as well as for an evaluation of prophylactic therapy Ventricular angiography cannot be used as a routine method and small mural thrombi will presumably not be detected by this method

Use of the ^{125}I labelled fibrinogen scanning technique for diagnosing deep venous thrombi has become fairly widespread in recent years With this technique Simmons et al (9) noted extremely high radioactivity counts over the precordial reference point in some AMI patients They suggested that this fibrinogen accumulation in the heart was likely to be associated with mural thrombosis Later using the same technique Warlow and Terry (10) described 4 patients from a total of 83 with AMI in whom the precordial radioactivity did not show the expected fall with time possibly due to intracardiac mural thrombi

The purpose of the present report is to analyse the significance of increased precordial radioactivity in patients treated for an AMI who had been given ^{125}I labelled fibrinogen for the detection of deep venous thrombi and who had received no anticoagulant treatment

PATIENTS AND METHODS

Eighty patients 59 men and 21 women (mean age 61 years range 45–94) who were treated in the CCU at Serafimerlasarettet Stockholm for a proven AMI were given approximately 100 μCi ^{125}I labelled fibrinogen (Radiochemical Centre Amersham) i v on the day of admission At least 4 hours prior to the injection the thyroid gland was blocked with 100 mg potassium iodide which also was given daily for the remainder of the hospital stay Criteria for admission diagnosis and the routines employed in the CCU have been presented elsewhere (3) The only criterion for the selection of patients was availability of ^{125}I labelled fibrinogen at the time of admission and

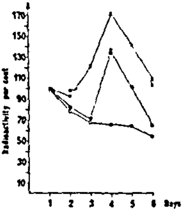


Fig 1 Precordial radioactivity curve of the type A pattern O—O=CR_1 × ×=CR_2 ×—×=CR_3 ●—●=CR_4

vival of at least 6 days None of the selected patients died within one month of injection of the fibrinogen and no patient had clinical evidence of peripheral embolization

Radioactivity was recorded daily for at least 6 days over four marked sites on the precordium corresponding to the four precordial leads CR_1–CR_4 All scans were performed by the same person with a Pitman 235 Isotope Localization monitor The decay curves of precordial radioactivity were classified as follows Type A Sustained rise in the precordial radioactivity amounting to at least 15% in one or more of the marked sites as compared with the first measurement (Fig 1) Type B Minor rise (<15%) or a flattened pattern without a continuous decrease in radioactivity in one or more of the marked sites (Fig 2) Type C Rapid and continuous decrease in precordial radioactivity in all recorded sites (Fig 3)

These decay patterns were compared in relation to various clinical and laboratory parameters The χ_2 test with Yates correction was used for testing the significance of differences of relative numbers

Patients developing pathological Q waves (8) in at least 2 leads were classified as having transmural infarctions and patients lacking such Q waves but with diagnostic enzyme curves were classified as having non transmural (subendocardial) infarctions Involvement of the apex region was suspected when Q waves appeared in a le 2 of the leads CR_4 CR_5 and CR_6

Definite pericarditis was considered to be present wh a pericardial rub was noted by at least 2 observers Pe carditis was suspected in patients with non ang al p and varying with respiration

The febrile reaction was grouped arbitrarily as fo 1) Minor maximum temperature below 38°C 2) Mod rate maximum temperature not exceeding 38 6°C Major maximum temperature exceeding 38 6°C

RESULTS

Twenty two (28%) of the patients had an increa in precordial radioactivity fulfilling the criteria f the type A decay pattern 15 (19%) had an in e mediate response of the type B pattern and 4 (54%) had a rapid decline in precordial radioa tivit corresponding to the type C pattern

The incidence of definite and suspected per carditis is shown in Table I When patients with d finite and suspected pericarditis were combine significant differences appeared Sixteen (73%) the patients with type A decay pattern had defi or suspected pericarditis compared with 8 (19%) those with type C pattern ($p<0.001$) In the gro with type B decay 4 patients (27%) had defin or suspected pericarditis which also differs sig ficantly from the findings in the type A gro ($p<0.05$)

The different types of infarction in relation precordial radioactivity patterns are presented Table II The incidence of transmural infarction patients with type A decay curves was sligh higher (64%) but did not differ significantly fro that in patients with a type C decay pattern (4 Patients with transmural infarction were furth analysed as regards the location of the infarctio but no differences were found (Table III) Ap

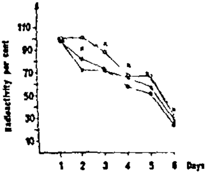


Fig 2 Precordial radioactivity curve of the type B pattern Symbols as in Fig 1

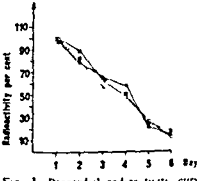


Fig 3 Precordial radioactivity curve of the type C pattern Symbols as in Fig 1

Table I *Clinical evidence of pericarditis in relation to precordial radioactivity decay patterns in 80 patients with AMI*

Type of decay	Pericardial rub (n=16)		Pericardial pain (n=12)		Total (n=28)		No pericarditis (n=52)	
	No	%	No	%	No	%	No	%
A	7	44	9	75	16	57	6	12
B	4	25	–		4	14	11	21
C	5	31	3	25	8	29	35	67

involvement was suspected in 7 (50%) of the 14 type A patients with transmural infarction compared with 5 (25%) of 20 patients with type C decay (N S)

Larger infarcts as evidenced by a maximum SGOT value exceeding 100 U/l were present in 19 (86%) of the type A 12 (80%) of type B and 19 (44%) of type C patients The larger infarctions were more common in patients with types A or B compared with type C patients ($p<0.01$ and <0.05 respectively) A minor febrile reaction was less common in patients with a type A decay pattern (23%) compared with type C patients (63%) ($p<0.05$) Those with type B decay pattern were intermediate in this respect

No differences were found as regards the incidence of past history of angina pectoris AMI hypertension congestive heart failure or maximum ESR reaction in connection with the acute illness in relation to pattern of radioactive decay The interval between onset of symptoms and injection of ^{125}I labelled fibrinogen was mainly dependent of the delay in admission and did not differ significantly between the groups

DISCUSSION

The true incidence of ventricular mural thrombi after AMI is not known since no accurate method is available so far for its diagnosis in vivo Since none of the patients died we cannot state for certain whether an increased precordial activity is found only in the presence of mural thrombi or if other factors may influence the results Conceivably an increased precordial radioactivity may also be caused by 1) fibrinous pericarditis secondary to AMI 2) coronary thrombus formation and 3) fibrin deposition within the myocardium associated with the inflammatory process following the necrosis The latter alternative seems unlikely since fibrin deposition is not detected microscopically in coagulation necrosis (2)

Pericarditis is a complication of transmural but not of subendocardial infarction The incidence of pericarditis in transmural infarction according to autopsy studies varies (1 6 7) but was found in approximately half of the patients in a recent study from this hospital (3) The presence of a pericardial rub was not found to be related to an increased precordial activity in the present study Thus it is not likely that it is the accumulation of fibrin over the pericardium that is a major factor accounting for an increase in precordial radioactivity On the other hand if patients with pain suggestive of pericardial irritation were added a significant over representation appeared among the type A decay curves However 5 patients with a pericardial friction rub lacked an increase in precordial radio-

Table II *Infarction types in relation to precordial radioactivity decay patterns in 80 patients with AMI*

Type of decay	Transmural (n=42)		Subendocardial (n=35)		Bundle branch block (n=3)	
	No	%	No	%	No	%
A	14	33	7	20	1	–
B	8	19	5	14	2	–
C	20	48	23	66	0	

Table III *ECG position of transmural infarction in relation to precordial radioactivity in 42 patients with AMI*

Type of decay	Anterior (n=25)		Inferior (n=17)	
	No	%	No	%
A	9	36	5	29
B	6	24	2	12
C	10	40	10	59

activity Thus the significance of pericardial fibrinous exudation due to secondary pericarditis in relation to precordial radioactivity remains uncertain but definitely does not adequately explain an increase in precordial radioactivity which is in agreement with the findings of Warlow and Terry (10) Although coronary thrombi will show evidence of radioactivity when fibrinogen is injected after the onset of an AMI (4) the amount of radioactive material is very small and would not be expected to give rise to an increased precordial count

The most likely explanation of the increased precordial radioactivity is the presence of ventricular mural thrombi as has been suggested previously (9 10) Mural thrombi in the left ventricle are found at autopsy more often in patients with transmural infarction than in those with subendocardial infarction and are frequently associated with infarction of the apex (3 5) In a recent autopsy study from our unit (3) mural thrombi were found in 40% of the patients regardless of infarction type which may be compared with the 28% incidence of type A decay curves in the present study Furthermore as in the autopsy study mural thrombi occurred in 51% of the patients with transmural infarction and in 13% of those with subendocardial infarction Thus although a difference in the incidence of mural thrombi in relation to infarction type and location might be expected there was no corresponding difference according to the type of precordial activity in the present study

Our findings are somewhat at variance with those of Warlow and Terry (10) since they found only 4 of 83 patients who did not show the normal decay in precordial levels of radioactivity These 4 patients all had clinical evidence of pericarditis and a mural thrombus was found at autopsy in 2 of them These authors point to 14 of the other patients as having clear evidence of pericarditis yet still showing normal decay of radioactivity They do however suggest that transient rises in activity are not uncommon and point to the necessity of a sustained rise for evidence suggesting mural thrombosis

The present study indicates that different patterns of precordial radioactivity decay curves are found in patients given ^{125}I labelled fibrinogen after an AMI It is likely that patients showing an increased precordial radioactivity have ventricular mural thrombi but we are at present unable to decide whether other conditions e g secondary fibrinous pericarditis may influence the results The use of other isotopes together with a gamma scintillation camera may be of help in solving this problem

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Prevalence of Hypercalcaemia in a Health Screening in Stockholm

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ABSTRACT A free health check, offered to 21 417 20–63 year-old employees of the Stockholm City and County Council in 1971–73, was accepted by 15 903 persons The examination included a multichannel chemical analysis of a single blood sample Serum calcium levels ≥11 0 mg/100 ml (2 75 mmol/l) and ≥11 1 mg/100 ml (2 78 mmol/l) were encountered in 3 9% and 1 1% of the population, respectively Among subjects below 50 years of age, the calcium concentration was significantly higher in males than in females This difference disappeared in older subjects, essentially because the calcium level decreased with advancing age in the men To a further investigation were invited 178 subjects with a single serum calcium registration ≥11 1 mg/100 ml (2 78 mmol/l) Of this group, 95 persons (53 4%) exhibited hypercalcaemia (HC) on repeated testing Twelve had been operated on prior to the actual follow up and found to have parathyroid adenomata Twenty subjects were on continuous treatment with diuretics of the thiazide type and seven had diseases that might induce HC (two had hyperthyroidism, two hypothyroidism, one sarcoidosis, one hypernephroma and one mammary carcinoma) In 56 patients the laboratory and physical examinations did not reveal any obvious cause for the HC except possible hyperparathyroidism (HPT) Eighty (84 2%) of the 95 HC subjects were women mostly over 50 years The 95 persons constituted 6‰ of the total number of health screened persons The highest prevalence, 13‰, was recorded for women aged 60–63 The prevalence of HPT in the total material was 3 6‰, which is higher than that found in several other studies This is based on surgical findings to date

Several attempts have been made to estimate the prevalence of hypercalcaemia (HC) on the basis of findings in hospitalized and ambulatory patients The results have varied as exemplified in Table 1 The discrepancies may have several explanations such as differences in age and sex of the populations studied selected materials differences in the limits used for defining HC and variable specificity of the analytical methods HC is associated with several disorders apart from hyperparathyroidism (HPT) e g certain malignant disorders sarcoidosis and hyperthyroidism It may also occur in patients treated with thiazides and thiazide like drugs The increasing use of such therapy in the management of hypertension and heart failure may influence the current prevalence of HC

An opportunity to estimate the prevalence of HC in a large and rather unselected population of the Stockholm area appeared when the Stockholm City and County Council started to offer its employees a health check service The present report summarizes the results obtained in that investigation during a period of two years

MATERIAL AND METHODS

The health check service Employees of the Stockholm City and County Council who are more than 50 years old have the benefit of a free medical health check every second year Those under 50 are offered a check every fifth year The examination comprises a physical and laboratory investigation including conventional chemical analyses of urine and blood samples (obtained at variable hours of the day and without restriction of dietary intake) an ECG and an X ray of the chest The medical history is obtained by means of a standardized questionnaire All data have been organized for computer analysis

The subjects Altogether 15 903 persons accepted a

Table I *Prevalence of hypercalcaemia (HC) in some reports of hospitalized (HP) or ambulatory patients (AP) and in health examined subjects (HE)*

	Study period	No of subj	HC Limit[a] (mmol/l)	HC Prevalence (%)	Serum calcium recordings per person	Reference
HP	1966–67	1 989	2 88	0 3	Single	Daughaday et al (10)
HP+AP	Not specified	4 000	2 88	2 2	Single	Reece (31)
HP (♀)	1967–68	509	2 75	2 9	Several	Boström and Wengle (5)
AP	1968–69	2 005	[b]	0 1	Several	Keating et al (21)
HP	1969–70	8 027	2 63	0 4	Single	Mays and Weakley (26)
HP	1968–71	3729[c]	2 70	1 4	Two	Kelstrup et al (23)
HE (♂)	*1973–74*	*6 048*	*2 63*	*0 3*	*Single*	*Ryckewaert et al (33)*
HE	1971–73	15 903	2 78	1 1	Single	The present study

[a] Registrations originally in mEq/l or mg/100 ml are recalculated to mmol/l [b] Specified with regard to sex and age
[c] Readmissions included

health check offered 21 417 employees in the period July 1971–July 1973 The oldest employees reached their 63rd birthday during the relevant year The offer during this two-year period was extended to all 50–63 year old employees and to about 40% of those under 50 The drop out was 25 7% for the whole series 25 1% for men 26 1% for women The lowest response (47% for males 51% for females) was recorded in the youngest (20–31 year old) group The sex and age of the participants are shown in Fig 1

The follow up Subjects with serum calcium levels ≥11 1 mg/100 ml (Auto Chemist recorded) were selected for a follow up at the Out patient Clinic of Serafimerlasarettet At this examination a medical history was taken which included detailed information about the family history medication past and present illnesses A physical and a laboratory investigation were performed again Blood specimens were drawn with minimal venous occlusion in the morning of three consecutive days with the subjects in recumbent position They had been instructed to fast overnight and to avoid milk and cheese during the preceding three days None was on any treatment with vitamins or calcium substitution Three persons who used antacid occasionally (combination of aluminium and magnesium hydroxide) were told to avoid this treatment during the examination period Two women were on hormonal treatment one with a low dose oestrogen oral contraceptive and one with oestrogen for menopausal symptoms The blood samples were analyzed for calcium phosphate magnesium albumin creatinine urate parathyroid hormone (PTH) thyroxine amylase alkaline phosphatases and Hb The study also included determinations of ESR cellulose acetate electrophoresis of the serum proteins as well as determination of the excretion of protein glucose and calcium in urine

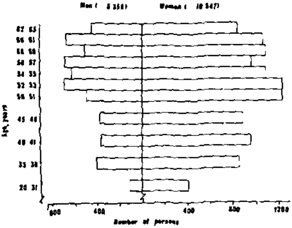


Fig 1 Age and sex distribution of the participants

Within six months subjects with repeated recordings of elevated serum calcium at the Out patient Clinic follow-up were hospitalized and studied under standardized dietary conditions During this period the daily intakes of calcium magnesium and phosphorus were about 800 350 and 800 mg respectively Kidney function tests were performed as well as X ray investigation of the urinary tract and of certain parts of the skeleton

Determination of serum calcium The serum analyses in the health screening program were performed using an Auto-Chemist recorder (AGA AB Stockholm Sweden) and the results were expressed in mg/100 ml The coefficient of variation was 1 6% (19)

Blood samples collected during the follow up were analyzed at the Chemical Department of Serafimerlasarettet During the first part of the study serum calcium was determined using the Technicon AutoAnalyzer (Technicon Instruments Corp Tarrytown New York USA) by a method as modified by Gitelman (12) The AutoAnalyzer was later replaced by Atomic Absorption Spectrophotometry AAS (Perkin Elmer Norwalk Connecticut USA) During the change over aliquots of identical blood samples were analyzed by both techniques Comparison of the results yielded the regression equation $y = 0.98x - 0.01$ in which the dependent variable y represented the result of AAS and the independent variable x the figure for the AutoAnalyzer expressed in mmol/l This comparison revealed that the AAS values were about 2% lower than the AutoAnalyzer values The latter results

were recalculated according to the equation above to achieve equivalence with AAS data. The accuracy of the latter method was within 0.01 mmol/l and the coefficient of variation was not allowed to exceed 1.5%.

At Serafimerlasarettet a serum calcium concentration of 2.70 mmol/l or more in at least two of three analyzed samples according to the AutoAnalyzer technique was defined as verified hypercalcaemia (VHC). This limit corresponded to 2.64 mmol/l when the analysis was performed according to the AAS technique. For the 14 subjects who were examined at other hospitals the definition of VHC was based on local criteria.

Other laboratory methods. Laboratory analyses were performed by routine methods. Technicon AutoAnalyzer and AAS were used in the determination of calcium in urine and magnesium in serum. PTH was determined at Stockholms Immunlaboratorium AB by a method reported by Almkvist et al. (2). X rays were taken of the long bones, hands, skull and urinary tract. Subperiosteal resorption was defined as described by Camp and Ochsner (7). Conventional statistical methods were used (3).

RESULTS

Serum calcium level

In the health screened subjects under 50 years the serum calcium concentration was higher in men than in women (Fig. 2), the means being 10.2 and 10.0 mg/100 ml respectively ($p<0.01$). The calcium level decreased with advancing age in the males but not in the females. In the subjects aged 50 or over

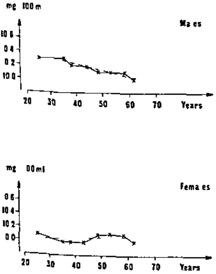


Fig. 2. Serum calcium in relation to age in the total series of 15 903 subjects. The curves are based on the means for each age group in Fig. 1 (corrected for the different sizes of the groups). The regression equations for males and females are $y = 10.4996-0.00736x$ ($r = -0.86$) and $y = 9.9733+0.00178x$ ($r = 0.15$), respectively.

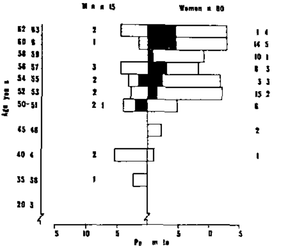


Fig. 3. Prevalence of verified hypercalcaemia (VHC) in women and men separated with regard to age. The figures next to the parentheses indicate the total number of subjects with VHC. The number of thiazide treated patients (■) is given within parentheses.

the mean serum calcium level (10.1 mg/100 ml) was similar for both sexes. Serum calcium equalled or exceeded 11.0 mg/100 ml in 620 of the 15 903 subjects (3.9%). A level of 11.1 mg/100 ml or higher was recorded in 178 persons (1.1%) who were offered a reexamination.

Prevalence

The follow up included all but six of the invited subjects. Four refused to participate, one had moved to another part of the country and one had died in a traffic accident. Fourteen subjects had been sent to other hospitals prior to the actual follow up by their own physicians on suspicion of HPT and were not reexamined by us. Two of them were considered to be normocalcaemic according to local criteria, while the other 12 had VHC. The subsequent operations revealed adenomata of the parathyroid glands in all 12 cases.

Of the 158 subjects examined at the Out patient Clinic of Serafimerlasarettet 84 (68 females, 16 males) had VHC. All but one had this finding confirmed on admission. The exception was a 61 year old male not on thiazide treatment who had asymptomatic HC. The remaining 83 subjects together with the 12 with VHC confirmed at other hospitals constituted 95 (6‰) of 15 903 persons.

As illustrated in Fig. 3, only six of the 95 subjects (three females, three males) were under 50 years. The occurrence of VHC was at least 5‰ in each

Table II *Serum calcium, serum phosphate and creatinine clearance in 83 subjects participating in the follow up at Serafimerlasarettet*

Mean range and S E M are based on the mean values of each case Range based on the individual observations is given within parentheses Group I=thiazide treated patients group II=patients with diagnoses as present d below group III=the remaining subjects with VHC

	No of subj	Serum calcium (mmol/l)			Serum phosphate (mg/100 ml)			Creatinine clearance (ml/min)		
		Mean	Range	S E M	Mean	Range	S E M	Mean	Range	S E M
Group I	18 ♀ 2 ♂	2 78	2 61–3 42 (2 54–3 42)	0 04	2 8	1 1–4 6 (1 0–5 9)	0 2	98	62–125 (60–132)	4
Group II										
Hyperthyroidism	2 ♀	2 80	2 77–2 82 (2 69–2 88)		2 6	2 1–3 1 (1 4–3 4)		126	125–127 (116–134)	
Hypothyroidism	2 ♀	2 63	2 56–2 70 (2 40–2 78)		3 5	3 3–3 7 (3 1–4 0)		95	92–98 (92–100)	
Sarcoidosis	1 ♀	2 64	2 64–2 64 (2 59–2 69)		3 1	3 1–3 1 (2 7–3 6)		138	138–138 (135–140)	
Malignant diseases (hypernephroma mammary carci noma)	2 ♀	2 67	2 67–2 67 (2 58–2 76)		3 5	3 1–4 0 (2 6–4 9)		140	138–142 (133–150)	
Group III	43 ♀ 13 ♂	2 75	2 59–3 08 (2 34–3 22)	0 02	2 8	2 1–3 4 (1 4–4 9)	0 1	97	39–147 (28–155)	5
Normal range			2 30–2 64			2 5–4 8			85–145	

age group among the women over 50 The average figure among women aged 50–63 was 10 3‰ The rate was highest 13‰ for the oldest women (60–63 years) The prevalence of VHC among men aged 50–63 was 2 9‰ and did not exceed 5‰ in any age group VHC was not encountered in the youngest subjects aged 20–31

In the 75 subjects who did not fulfill the criterion for VHC at the follow up at Serafimerlasarettet the mean serum calcium level based on means of individual cases ranged from 2 35 to 2 69 mmol/l Means of 2 64 to 2 69 mmol/l were recorded in four subjects amongst whom one was suffering from myeloma and another from Hodgkin s disease We recently learned that another woman of these 75 subjects was operated on at another hospital for parathyroid adenoma

Manifestations and laboratory findings

Clinical and laboratory findings in the 83 subjects with VHC who were reexamined at the follow up at Serafimerlasarettet are presented in Tables II and III

Twenty persons on continuous treatment with thiazides or thiazide like drugs (clopamide chlor thalidone) because of hypertension (18 cases) and peripheral oedema not related to heart liver or re nal failure (two cases) were referred to as group I In this group one woman in addition suffered from carcinoma of the uterine cervix After the reexami nation the thiazides were withdrawn and if neces sary replaced by other drugs The patients were subsequently followed at regular controls and to date 14 of them have been subjected to neck explo ration because of persistent HC Parathyroid ade nomata were revealed in all instances This part of the study will be reported in detail in a forthcoming paper

Of the subjects with VHC not treated with thiazides two suffered from hyperthyroidism two from hypothyroidism and three others from sarcoid osis hypernephroma and mammary carcinoma respectively altogether seven females (group II) After treatment the patients with hyperthyroidism hypothyroidism and sarcoidosis normalized their serum calcium

The remaining 56 patients with VHC were in cluded in group III Hypercalcaemic causes other than HPT were excluded as far as possible Most of these subjects considered themselves to be in good health Some characteristic disorders and symp toms that could be related to HC and HPT in this group will be analyzed in a comparative study with a control group matched for sex and age in a forth coming paper

In group III Hb albumin amylase thyroxine

Table III *Some data recorded at the follow up of 83 subjects with VHC*

Registrations of serum phosphate serum magnesium serum alkaline phosphatases urinary calcium and creatinine clearance are means of repeated recordings Mean and S E M based on mean values of each case are presented Grouping of patients as in Table II A f =abnormal findings

	Normal range	Group I (18 ♀ 2♂) A f	Group I Mean	Group I S E M	Group II (7 ♀) A f	Group III (43 ♀ 13 ♂) A f	Group III Mean	Group III S E M
Serum phosphate (mg/100 ml)	2 5–4 8	↓ 6	2 8	0 2	–	↓15	2 8	0 1
Serum magnesium (mmol/l)	0 80–1 00	↓10	0 79	0 02	–	↓11	0 82	0 02
Serum PTH (ng/ml)	1 1–2 5	↑ 2	2 0	0 1	–	↑18	2 4	0 1
Serum alkaline phosphatases (mU/ml)	<40	↑ 1	23	2	–	↑ 2	23	1
Creatinine clearance (ml/min)	85–145	↓ 3	98	4	–	↓ 9	97	5
Renal concentrating capacity		↓ 6			↓ 1	↓16		
Urine calcium (mmol/d)	1 4–5 5	↑ 8 ↓ 1	5 6	0 6	↑ 3	↑35	7 5	0 5
Skeletal X ray (subperiosteal resorption)		1			–	12		
Renal calculi (X ray verified)		2			–	8*		
History of renal calculi (no X ray findings at present)		2			–	8		

* Including one case with calcified deposits in parenchyma in addition to concrement

and urate were within normal limits in all instances No proteinuria was revealed glucosuria was found in one woman known to be suffering from diabetes mellitus Serum electrophoresis did not show any evidence of a monoclonal fraction Using the upper limits outlined by Bottiger and Svedberg (6) ESR was elevated in two subjects but was normalized later Serum creatinine ranged from 1 3 to 1 4 mg/100 ml in two subjects both of whom had normal creatinine clearance it was reduced in nine subjects and 16 had decreased renal concentrating capacity

Urinary calcium and PTH were elevated in 35 and 18 persons respectively Serum phosphate was below normal in 15 subjects and two had a slight elevation of alkaline phosphatases (X ray showing subperiosteal resorption in one case) Magnesium in serum was subnormal in 11 subjects

Subperiosteal resorption was observed in 12 and renal calculi in eight subjects Eight other persons without X ray evidence of renal concrements had a well documented history of previous episodes of renal calculi that had been verified by X ray

DISCUSSION

The use of serum calcium as a screening test in health control surveys accentuates the need of accurate knowledge of the concentration and range of serum calcium in healthy subjects The importance of taking sex and age into account is clearly demonstrated by the present results Among subjects under 50 the mean serum calcium level is significantly higher in males The calcium concentration decreases in men with advancing age and the mean values for males and females over 50 years are equal Similar results have been reported by e g Keating et al (22) in a smaller population of healthy Americans

The ranges for serum calcium in healthy persons vary with the specificity of the analytical methods used (35) As demonstrated by the present health check survey even a minor change in the limit selected for defining HC is sufficient to have a marked effect on the results If a serum calcium concentration of 11 1 mg/100 ml is used as the upper limit the prevalence of HC amounts to 1 1% 3 9% have a concentration of 11 0 mg/100 ml or higher The selection of the former level in this study was dictated purely by practical reasons since we were primarily unable to reexamine as many as 620 subjects Considerable errors may arise moreover when calculations are based on analysis of only one serum sample Thus in accordance with the results of similar studies (23 28 36) only about half of the subjects showed persistent HC at a follow-up with

repeated tests At the same time screening of HC based on a single recording initially, will lose a number of patients who would have been diagnosed as VHC if the investigation had been performed with repeated tests

For all the subjects studied in the health check the prevalence of VHC was 6‰ (95 of 15903 subjects) The prevalence of VHC was higher in females than males and highest among the oldest women in agreement with previous reports (30)

The diagnosis of HPT is supported to a variable extent by findings such as a subnormal level of phosphate low serum magnesium elevated concentration of serum PTH alkaline phosphatases above normal decreased creatinine clearance reduced renal concentrating capacity, increased urinary calcium excretion radiological signs of subperiosteal resorption and actual findings of or well documented histories of previous episodes of renal calculi (1 17 20 24, 30) In group III three or more of these parameters were scored as positive in 24 subjects Of the other 32 six showed none 11 one and 15 two of the parameters mentioned above Although no specific combination of findings is found to be predictive of HPT some of the manifestations occur very frequently as presented in Results and Table III e g subnormal phosphate low magnesium in serum elevated level of PTH increased urinary calcium excretion decreased creatinine clearance or renal concentrating capacity subperiosteal resorption and renal calculi

Altogether 59 patients participating in the present health control survey have been subjected to neck exploration Included in this group are the 13 patients operated upon in other hospitals and 46 of the subjects with VHC reexamined at Serafimerlasarettet (14 thiazide and 32 non thiazide treated subjects) Parathyroid adenomata were found in 57 patients bringing the prevalence of HPT to 3 6‰ (57 of 15903 subjects) Since some of the subjects with VHC are still awaiting operation it seems likely that the number of patients with findings of parathyroid adenomata will increase VHC disappeared in all cases postoperatively

The prevalence of HPT of 3 6‰ is higher than e g those of 0 2–3 3‰ previously reported for adult populations from Europe and the USA (4 8 15 16 18 21 23) It appears then that HPT is the most common cause of HC in an unselected population Several surveys of HC in hospitalized patients show a higher frequency of malignant diseases (13 26 28)

In conclusion the present study demonstrates that screening of serum calcium in health control surveys is of value for the detection of hypercalcaemic patients many of whom may be suffering from HPT However it is also evident that analysis of a single serum sample provides only approximate information on the prevalence of HC in general and HPT in particular The reasons are several Firstly it is difficult to define a biologically meaningful lower limit of HC Secondly the serum calcium level tends to fluctuate spontaneously (9 11 27 37 38) which makes correct identification of HC difficult Thirdly accidental errors in the technique of collecting blood samples may result in falsely abnormal values Especially cases of borderline HC call for carefully standardized methods (34 39) Fourthly an increasing number of normocalcaemic patients with HPT has been reported in recent decades (14 25 29 32)

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BOOK REVIEWS

Primary pulmonary hypertension Report on a WHO meeting Edited by S Hatano and T Strasser 45 pp 10 Sw fr World Health Organization Geneva 1975

This is a report on a meeting which WHO convened in 1973 to review various aspects of primary pulmonary hypertension and to define related problems requiring further investigation There are chapters on control and pharmacology of the pulmonary circulation and on classification nomenclature morphology etiology pathogenesis clinical features diagnosis and epidemiology of primary pulmonary hypertension

Embracing these topics in 45 pages the report forms a concentrated guide book rather than a manual of primary pulmonary hypertension There are broad outlines of the major features problems and prospects of the field but not much detailed description or analysis Much in agreement with the aim of the meeting the book serves well as a basis for further studies

Erik Trell Malmo Sweden

The Acute Coronary Attack By J F Pantridge A A J Adgey J S Geddes and S W Webb 141 pages £6 Pitman Medical Publishing Co Ltd Tunbridge Wells Kent 1975

This book is the result of nearly ten years experience with a mobile CCU in Belfast Between 1966 and 1970 3861 calls were managed by the unit Forty two per cent had evidence of myocardial infarction and this vast experience of handling MI patients during the very early stage has convinced Pantridge and co-workers that this form of pre hospital treatment is an important part of the whole coronary care A large part of the book is devoted to arrhythmias during the early phase and here a lot of theoretical and practical problems are put forward such as that a chest thump can convert a ventricular tachycardia to both sinus rhythm or ventricular fibrillation. A chest thump is also shown to convert ventricular fibrillation to sinus rhythm The authors main issue is that the early coronary care reduces infarct size and thereby prevents the development of shock and pump failure The falling incidence of shock in modern CCUs is probably an effect of many therapeutic schemes where the mobile CCU is a small part Although the mobile CCU was initiated in Belfast in 1966 only 9 such units operate in Britain In an appendix it is shown that only about 50 are in function all over the world and most of these are staffed with paramedical personnel For clinicians and administrators who are involved in the management of early coronary care the book contains a lot of valuable information

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Extensive Nodular Infiltration of Extra-osseous Tissues in Human Myelomatosis

A Case Report

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ABSTRACT The autopsy findings of a woman with myelomatosis diagnosed half a year before her death are described She had an IgG-λ myeloma which initially responded to treatment However, subsequently she developed a condition characterized by massive soft tissue involvement with increased number of plasma cells in peripheral blood Rounded hard myeloma infiltrates were disseminated through the body only sparing the adrenal glands and intra cranial structures

Extra osseous infiltration of myeloma cells at autopsy is reported in approximately 70% of cases with multiple myeloma (3 9) Lymph nodes spleen liver and kidney are frequently involved Metastatic spread to adrenals pancreas lungs and heart etc is less common (4 5) A moderate number of plasma cells in the peripheral blood is a common finding in patients with myelomatosis especially in advanced disease (11) A few patients develop a clinical pattern characterized by hepatosplenomegaly and WBC exceeding 15000 with plasma cells ranging from 10 to 90% These patients often suffer a fulminant course with many features similar to acute leukemia The diagnosis of plasma cell leukemia is designated to these patients (10)

Subleukemic variants of myelomatosis have been described with a clinical course that is less fulminant than plasma cell leukemia (1) These patients have a significantly increased number of plasma cells in the peripheral blood although rarely exceeding 10000/mm³ A patient with an IgG λ myeloma of short duration with light chain excretion running a fulminant course is described below

CASE REPORT

A 61 year-old woman was admitted to hospital in May 1974 because of fatigue and a serum M-component. The past history included an episode of renal calculi treated conservatively in 1971 and bronchopneumonias in 1972 and 1973 The family history was non contributory Laboratory investigations ESR 46 mm/h Hb 12 6 g/100 ml platelet count 174000/μl WBC 3600/μl with a normal differential count Immunoelectrophoresis of serum revealed a monoclonal peak composed of IgG λ Immunoelectrophoresis of concentrated urine showed an M component consisting of free λ light chains Quantitation of serum immunoglobulins IgG 6 3 IgA<0 025 IgM<0 03 g/100 ml

During the out patient investigation she became acutely ill with fever diarrhea and dyspnea and was therefore admitted to the emergency ward A chest X ray showed pulmonary infiltrates pulmonary vascular congestion as well as interstitial edema Laboratory investigations Hb 13 8 g/100 ml WBC 21000–4000/μl with initially 90% toxically granulated neutrophils She had also a transitory renal insufficiency with a serum creatinine of 5 2 mg/100 ml Serum calcium was slightly increased 2 63 mmol/l Bone marrow biopsy showed 95% plasma cells of varying maturity X ray examination of the skeleton failed to reveal any abnormalities The patient was considered to have a plasma cell myeloma (IgG λ type) Melphalan (0 25 mg/kg/day) and prednisolone (2 mg/kg/day) for four days was instituted to be repeated every sixth week The patient was discharged in the beginning of June In the middle of the month she again developed bilateral bronchopneumonias which promptly responded to antibiotics Her second melphalan–prednisolone course was started in July by which time her monoclonal immunoglobulin peak had decreased the total amount of IgG being 1 9 g/100 ml The patient s general condition improved continuously and she returned to full working capacity

However two weeks after the fourth course of treatment she began to complain of epigastric pains and heart burns An X ray of the stomach on Nov 21 showed an ulcer on the minor curvature which was treated conserva

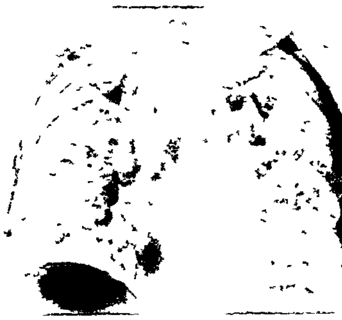


Fig 1 X ray of the lungs showing diffusely scattered nodular infiltrations corresponding to the findings of Fig 2*a*

tively Her condition deteriorated however and she was readmitted at the end of Nov On admission she was pale exhausted and vomited persistently There was a marked epigastric tenderness Hb 10 8 g/100 ml WBC 3 500/μl with 73% mature granulocytes 13% lymphocytes No plasma cells were found in the peripheral blood Platelet count 145 000/μl Parenteral nutrition and a gastric tube with slight suction were applied with some improvement Gastroscopy confirmed the ulcer detected earlier No bleeding was noted and biopsies revealed no malignant infiltration. From Dec 8 the patient s condition deteriorated rapidly She developed fever and ascites and became oliguric with an increase in serum creatinine A chest X ray showed generalized infiltrates of uncertain origin (Fig 1) The ascitic fluid contained numerous immature plasma cells and lymphocytes Treatment with diuretics antibiotics and chemotherapeutics supplemented with steroids and parenteral fluid therapy was ineffective The renal insufficiency progressed and the bowel became more extended WBC had increased to 12 200/μl with 2 200 lymphocytes 500 plasma blasts and 1 200 plasma cells platelet count 19 000/μl and Hb 8 6 g/100 ml The patient died on Dec 17 1974 having been unconscious for four days

At autopsy rounded hard myeloma infiltrates were disseminated through the body only sparing the adrenal glands and intracranial structures The infiltrates were yellow white and of varying size the largest resembling a pea. Both the parietal and the visceral pleural folds showed densely spread metastases Tumor growth was found along the bronchial tree and its vessels and diffusely seeded into the lungs (Fig 2*a*) Both folds of the pericardium exhibited myeloma infiltrates Furthermore these rounded tumors were found firmly attached to the endocardium of all cardiac chambers but the left atrium (Fig 2*b*) The heart weighed 330 g The peritoneum and the abdominal organs were covered with myeloma infiltrates (Fig 2*c*) The lesions were confluent in the pancreas which was almost entirely replaced by malignant growth (Fig 2*d*) The liver was of ordinary size but packed with rounded grey white infiltrates The spleen weighed 160 g and showed only metastases in the hilus. The kidneys displayed diffusely scattered infiltrates At microscopical examination the clearly defined rounded myeloma infiltrates were found to intersperse diffusely among morphologically unchanged tissue The cells of the infiltrates were small with a moderately varying morphology The nucleus was often placed eccentrically The histologic picture was in full agreement with the diagnosis of a low differentiated small cellular plasma cell myeloma.

DISCUSSION

Plasma cell leukemia has features in common with myelomatosis Thus the sex and age distribution does not differ from patients with myelomatosis (8) The immunoglobulin abnormalities are similar to those of multiple myeloma (10) The symptomatology of patients suffering from plasma cell leukemia or myelomatosis is essentially the same although the former often runs a fulminant course An increased number of plasma cells in the peripheral blood is sometimes seen in advanced myelomatosis This release of plasma cells into the blood stream might be a passive phenomenon caused by compression fractures in combination with extensive bone lysis In plasma cell leukemia the number of plasma cells is increased by definition and the

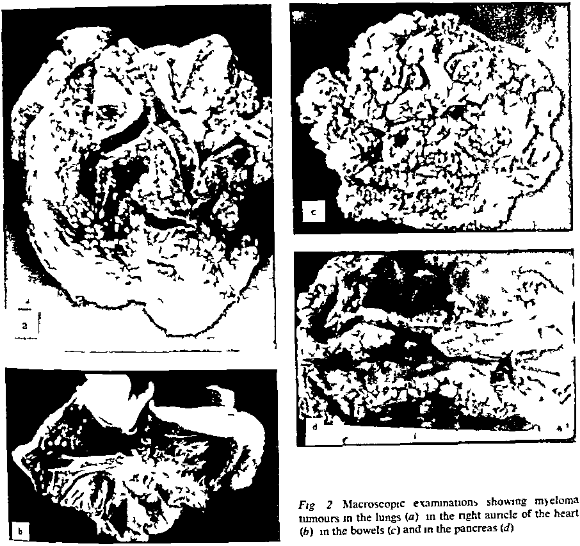


Fig 2 Macroscopic examinations showing myeloma tumours in the lungs (*a*) in the right auricle of the heart (*b*) in the bowels (*c*) and in the pancreas (*d*)

extensive soft tissue involvement is explained by hematogenous metastatic spread (1)

The peripheral blood of patients with plasma cell myeloma contains an increased number of bone marrow derived (B) lymphocytes In untreated patients most of the circulating B lymphocytes carry surface immunoglobulin with the same antigenic characteristics as the monoclonal immunoglobulin in serum (7) As B lymphocytes can develop towards plasma cells (2 6) these lymphocytes are thought to be progenitors of the malignant plasma cells Consequently multiple myeloma may be regarded as a disseminated (leukemic) disease with malignant cells in the bloodstream clearly indicating the possibility of metastatic spread in patients lacking peripheral plasma cells The autopsy of this case showed an intense diffuse and nodular infiltration by malignant cells of most organs which is a common finding at autopsy of patients with plasma cell leukemia but not characteristic of myelomatosis (10) However also plasma cells of varying maturity were present terminally in the blood but not to the extent usually seen in plasma cell leukemia (10) The course of the disease during the last weeks was similar to that of acute leukemias Hence this patient had all criteria of a multiple myeloma from the onset but at the time of her death she had developed many subleukemic signs Sometimes the terminal leukemic stage of multiple myeloma may resemble plasma cell leukemia but the widespread infiltrations encountered in this case have not been described It is therefore conceivable that multiple

myeloma and plasma cell leukemia may represent different clinical manifestations of a common malignant entity

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Prolonged Symptoms of Brain Dysfunction—Adverse Effect of Levodopa

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ABSTRACT Symptoms of brain dysfunction occurred in a 67 year-old woman with idiopathic parkinsonism on treatment with levodopa The adverse effect reappeared in a more severe and prolonged form when she was treated one year later with levodopa in combination with the peripheral decarboxylase inhibitor Ro-4-4602 The symptoms were associated with a markedly altered level of HMPG (a noradrenaline metabolite) in the cerebrospinal fluid

After 10 years of clinical experience with levodopa in idiopathic parkinsonism no serious adverse effects have been reported The more recent combination treatment with peripherally acting decarboxylase inhibitors is also considered to be safe and free from adverse reactions (3) Thus the incidences of peripheral side effects such as gastrointestinal disturbance cardiac arrhythmias and postural hypotension are decreased in relation to the therapeutic effect However the central side effects of levodopa on mental disturbances are not reduced by combination treatment The more favourable ratio between therapeutic and side effects is most likely due to an increased and prolonged biological availability of levodopa in the brain (1)

Here we describe a patient with idiopathic parkinsonism developing prolonged somnolence pronounced rigidity and severe tremor during treatment with levodopa and decarboxylase inhibitor The symptoms did not subside completely until 3 months after the treatment had been discontinued

CASE REPORT

The patient was a 68-year old woman with symptoms of essential parkinsonism since the age of 60

The symptoms were typical including symmetrical bilateral tremor bradykinesia and cog wheel rigidity For several years she was treated with orphenadrine (Disipal®) with no side-effects In 1971 levodopa (Dopastral®) was substituted for orphenadrine in a dose of 1 2 g/day After about 1 year of levodopa therapy she suddenly developed intense tremor somnolence slurred speech and became unable to walk Temporarily an extensor plantar response occurred on the left side

Discontinuation of levodopa medication was followed by a gradual recovery over two weeks During the following year she was treated with amantadine hydrochloride (Symmetrel®) 200 mg daily which was well tolerated A few attempts to combine this treatment with benzhexol hydrochloride (trihexiphenidyl Pargitan®) had to be discontinued due to spells of confusion

On readmission to this hospital in 1973 the disease had become fairly severe She could not perform any house work needed help to dress and could feed herself only with a spoon Due to retropulsion she could not walk without support The plantar response was slightly extensor on the left side Apart from parkinsonian symptoms the physical examination was unremarkable

A new attempt was made to treat the patient with levodopa this time combined with the peripheral decarboxylase inhibitor Ro-4-4602 The patient was admitted to this clinic and all medication was discontinued with the exception of 2 5 mg dihydroergotamine (Orstanorm®) b i d Levodopa combination therapy started 9 days later with daily doses of 100 mg and 25 mg levodopa and the inhibitor respectively

During the first two days she had short episodes of confusion and nausea. Thereafter the medication seemed to be well tolerated The dose was increased by 100 mg levodopa and 25 mg decarboxylase inhibitor every 4 days On the 8th day of treatment when the daily dose was increased to 300 mg levodopa and 75 mg decarboxylase

inhibitor a slight increase of the tremor was noted On the following day the body temperature rose to 38 8 C concomitant with markedly increased parkinsonian symptoms including facial tremors She was deeply somnolent and the arms were kept extended and internally rotated

The BP was normal Pupils were of normal and equal size and reacted well to light There was an extensor plantar response on the left side but this had been present to a lesser extent even before treatment Otherwise the general physical and neurological examinations showed normal results EEG was diffusely slowed but showed no focal abnormalities Brain scan was normal A right sided carotid angiogram was normal except for a slightly delayed circulation in the frontal region Laboratory studies including Hb counts of WBC RBC and platelets ESR fasting blood glucose serum electrolytes S ASAT S ALAT urine protein sugar and acetone and cerebrospinal fluid protein and cell count were normal

No obvious improvement was noted for 3–4 weeks The tremor was controlled to some extent by 5–10 mg diazepam The patient was bedridden and had to be fed parenterally for almost a month During this time she was persistently somnolent answering questions by single but adequate words The body temperature was about 38 C for 4 weeks and then slowly decreased to normal values within 6 weeks The neurological symptoms diminished slowly It was not until 3 months later that she had recovered to her pretreatment condition

DISCUSSION

HMPG (4 hydroxy 3 methoxy phenylethylen glycol) and its sulphate conjugate are the major metabolites of noradrenaline in the central nervous system of man (4) Total HMPG in our patient was determined in lumbar cerebrospinal fluid by mass fragmentography (5) No attempts were made to measure the major dopamine metabolite homovanillic acid (HVA) since L-dopa treatment leads to falsely elevated HVA levels In a sample taken three days after discontinuation of levodopa combination therapy the HMPG level was 347 pmol/l After 12 and 14 weeks when clinically improved she had HMPG levels of 16 pmol/ml and 80 pmol/ml respectively The HMPG levels in CSF from untreated patients with Parkinsons disease are reported to be 76±13 pmol/ml (2) These analyses thus reveal that during the acute stage of the adverse reaction to levodopa the HMPG level was markedly elevated whereas after three months the level was low to return to normal range

COMMENT

It seems likely that the reaction observed in this patient was an adverse reaction to levodopa since it occurred on two occasions when levodopa treatment was attempted and the symptoms disappeared when levodopa was discontinued Levodopa is indicted rather than the decarboxylase inhibitor since the latter drug was not given in the first levodopa treatment period in 1971 The reaction was worse following the 1973 treatment period when levodopa was given together with decarboxylase inhibitor which increases the biological availability of levodopa in the central nervous system (1) Any comments on the mechanism behind this adverse drug reaction must be speculative However the involvement of brain catecholamines seems likely in view of the HMPG findings

With this case report we wish to alert our colleagues to the possibility of serious brain dysfunction as an adverse reaction to levodopa therapy

ACKNOWLEDGEMENT

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Immunoreactive Insulin in Portal and Hepatic Venous Blood in Patients with Insuloma

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ABSTRACT The insulin level has been determined simultaneously in portal and hepatic venous blood in four patients with insuloma before and after administration of glucose and tolbutamide Three patients displayed a higher insulin level in hepatic than in portal blood although no hepatic metastases could be detected by radiologic examination In contrast portal insulin concentrations always exceeded hepatic in four control patients investigated in a similar way The implications of these results are discussed

It is generally accepted that most neoplasms of the islets of Langerhans are benign adenomata About 20% of these tumors are insulin secreting (7) and approximately 10% of all cases of insuloma are malignant (7 9) Generally metastases from insulomas resemble in structure the primary tumor (7) Accordingly it seems reasonable to assume that some of the metastases might be insulin secreting The most common site for metastases is the liver (1 7) Therefore in an attempt to find out whether insulin secreting hepatic metastases were present or not in four patients with insuloma we followed the insulin concentration simultaneously in portal and hepatic venous blood before and after β-cell stimulation Four patients with various other diseases catheterized for diagnostic purposes (portal venography and manometry) were investigated in a similar way and served as controls

MATERIAL

Insulomas

Four non-obese women aged 47–67 years were investigated All had had short attacks of unconsciousness relieved by i v glucose Hypoglycemia had been recorded in all on several occasions In three of the patients (nos 1–3) hyperinsulinemia had been found in response to oral glucose or i v tolbutamide whereas one patient (no 4) never displayed hyperinsulinemia after similar stimulation None of the patients had had symptoms or signs indicating gross liver impairment (Table I) Moreover neither hepatic metastases nor portal systemic communications were found in any of them when examined by portal venography and hepatic arteriography A single pancreatic β-cell tumor was found at operation in patients 2 and 4 Two β-cell tumors were found in patient 3 one located to the head of the pancreas the other to a lymph node behind the pancreas Patient 1 has not yet accepted surgical exploration of the pancreatic gland

Controls

One 50-year-old woman and three men aged 40–70 years with various diseases (Table I) were investigated All were non-obese without glucosuria and had no family history of diabetes None had portal systemic communications

METHODS

Blood glucose was determined enzymatically by a glucose oxidase method using commercial reagents (Glox® Kabi Stockholm Sweden)

Immunoreactive insulin (IRI) in serum was assayed by a double antibody procedure essentially as described by Soeldner and Slone (13) Porcine insulin (10×crystallized) was used for immunization All insulin concentrations were determined by reference to a standard of 2×crystallized human insulin (obtained from Dr J Schlichtkrull Novo Research Institute Copenhagen Denmark) The error of the method calculated from 40 duplicate determinations according to the formula $\sqrt{\Sigma d^2/2n}$ was ±0 8 μU/ml at insulin concentrations between 6–15 μU/ml ±1.3 μU/ml at concentrations between 15–50 μU/ml ±2 8 μU/ml at concentrations between 50–80 μU/ml and ±4 2 μU/ml at insulin values between 80–220 μU/ml

Table I *Laboratory data and diagnoses*

Case no	Sex	Age (y)	Bilirubin (mg/100 ml)	Alk phosph (U/100 ml)	GOT (U/ml)	GPT (U/ml)	Po pressure (mm H_2O)	Diagnosis
1	♀	60	0 5	5 8	27	12	–	Insuloma
2	♀	47	0 8	4 0	6	30	100	Insuloma
3	♀	67	0 3	3 0	27	26	130	Insuloma
4	♀	54	0 4	3 3	25	30	–	Insuloma
5	♀	50	0 6	8 2	32	22	150	Hodgkin s disease
6	♂	70	0 9	43 0	81	60	–	Carcinoma of the papilla of Vater
7	♂	61	12 0	55 0	63	79	200	Hepatic cirrhosis
8	♂	40	0 7	6 0	20	7	190	Abdominal observation
Normal values			<1 2	<10 0	<40	<35	<150	

Catheterization of the portal vein was performed either by a transumbilic (4 20) or by a transhepatic technique (18 19) The transumbilic method was used preoperatively in all insuloma patients and in two controls (nos 5 and 8) The portal vein was reached transhepatically when patient 3 was reinvestigated seven months post operatively and also in the control patients 6 and 7 In all cases the tip of the catheter was placed in the common portal vein under fluoroscopic control and a slow drip of heparinized saline kept the catheter patent Both trans umbilic and transhepatic catheters could be left for a few days without any essential discomfort for the patients who were able to move freely in the ward and eat regular hospital meals At least one day always elapsed between the catheterization procedures and the experiments On the day of the experiment a catheter was also inserted into a hepatic vein through one of the femoral veins and a slow drip of saline was attached to the catheter

All experiments were performed after an overnight fast with the patients resting in a supine position In all patients except no 3 glucose ingestion (100 g) preceded an i v injection of tolbutamide (20 ml 5% Rastinon® Hoechst Frankfurt/Main West Germany) by 30–60 min Patient 3 was given an oral glucose load (100 g) pre operatively Postoperatively in this patient glucose ingestion (100 g) was immediately followed by an i v injection of tolbutamide (20 ml 5% Rastinon) and 100 min later by an i v infusion of glucose (25 g)

Blood samples for insulin and glucose determinations were drawn simultaneously from portal and hepatic veins before and after the various loads Student s *t* test was used for statistical evaluation

RESULTS

Mean fasting glucose concentrations (±S E M) in portal (MFPG) and hepatic venous blood (MFHG) did not differ significantly in the four insuloma patients (42±10 vs 44±9 mg/ml) In the control group, however MFHG was significantly higher than MFPG (93±1 vs 83±3 mg/100 ml $p<0.05$) In neither group did mean fasting portal IRI differ significantly from mean fasting hepatic venous IRI (insulomas 17±5 vs 12±3 μU/ml controls 22±7 vs 6±2 μU/ml)

A higher insulin concentration in hepatic than in portal blood was found after β cell stimulation in three insuloma patients (nos 1–3) when inves tigated preoperatively (Table II) In one of them (no 3) this finding was even reproduced postopera tively In the fourth insuloma patient (no 4) as well as in all the controls hepatic venous IRI never exceeded portal IRI

DISCUSSION

In three out of four patients with insulin secreting β cell tumors a higher insulin level in hepatic venous blood than in portal blood was found after β cell stimulation Since malignant β cell tumors often metastasize to the liver (1) a higher insulin concentration in hepatic venous blood than in portal blood might point to the existence of insulin secret ing metastases in the liver However hepatic meta stases could never be visualized in any of our pa tients by portographic or arteriographic examina tion

Although it is conceivable that very small hepatic metastases escaped radiological detection other explanations for the inverse relationship between portal and hepatic venous IRI must also be taken into account One is incomplete mixture of blood in the portal vein This has recently been observed in anesthetized dogs after infusion of insulin via a tributary of the portal vein (5) In awake patients however Strandell et al by using the ^{133}xenon dilution technique generally obtained adequate

Table II *Insulin and glucose concentrations before and after β-cell stimulation*

PIC=portal insulin concentration (μU/ml) HIC=hepatic venous insulin concentration (μU/ml) PGC=portal glucose concentration (mg/100 ml) HGC=hepatic venous glucose concentration (mg/100 ml) OG=oral glucose (100 g) IVG=i v glucose (25 g) IVT=i v tolbutamide (1 g)

Case no		OG		+IVT				
		0	30	60	62	67	70	80
1	PIC	14	90	178	368	422	370	348
	HIC	6	64	94	390	520	580	470
	PGC	70	139	170	163	153	140	109
	HGC	70	128	153	152	134	134	103
		0	35	30	32	37	40	
2	PIC	12	56	78	654	644	520	
	HIC	20	74	94	588	420	354	
	PGC	30	70	99	138	135	135	
	HGC	30	66	84	87	110	110	

Case no		OG					
		0	30	60	90	120	180
3	PIC	36	115	168	96	14	12
	HIC	8	110	288	68	44	16
	PGC	20	61	100	87	90	99
	HGC	33	54	104	100	99	103

Case no		OG+IVT					+IVG			
		0	12	17	20	70	100	130	140	155
3[b]	PIC	6	800	700	610	48	20	282	186	110
	HIC	8	680	720	660	28	414	1 020	1 160	500
	PGC	61	119	118	93	22	44	305	200	212
	HGC	61	111	113	89	19	45	322	224	215

Case no		OG		+IVT				
		0	30	50	52	57	60	65
4	PIC	16	50	48	108	86	90	96
	HIC	16	20	30	88	70	76	56
	PGC	30	121	167	167	167	179	167
	HGC	27	113	167	167	167	170	183
		0	30	60	62	67	70	90
5	PIC	31	108	150	220	204	320	215
	HIC	10	74	142	185	132	185	192
	PGC	74	177	190	190	194	196	206
	HGC	89	172	189	196	189	198	205
		0	30	60	62	67	70	90
6	PIC	27	48	46	104	107	98	152
	HIC	7	15	27	58	83	71	68
	PGC	88	212	279	307	307	305	281
	HGC	95	204	271	283	292	293	294
		0	30	60	62	67	70	90
7	PIC	2	40	43	181	151	127	173
	HIC	1	17	27	51	55	64	63
	PGC	88	145	162	174	172	183	168
	HGC	95	150	163	174	171	165	166
		0	30	60	62	67	70	90
8	PIC	29	112	217	345	380	530	380
	HIC	5	36	54	245	250	290	320
	PGC	82	140	180	167	176	158	124
	HGC	92	136	164	169	169	147	120

[a] Preoperatively [b] Postoperatively

mixing of xenon in the portal vein (14 15) Well mixed portal blood has also been found in anesthetized monkeys (6) But even if portal flow lamination does sometimes cause a lower insulin concentration in portal than in hepatic venous blood it remains to be explained why this would occur in the patients with insuloma only and not in any of the controls Perhaps streaming could be more important in insuloma patients because most of the insulin would be liberated from a single site in these patients Such a hypothesis can be neither rejected nor supported since investigations within this field have never been carried out in man before It has been shown however as a result of studies in dogs using labeled insulin that the vascular endothelium may adsorb insulin which can later be displaced into the bloodstream by the injection of unlabeled insulin or small amounts of glucose (11) Furthermore in vitro studies in rats have indicated that immunoreactive insulin may remain unaltered during reversible binding to liver cell membranes (2) According to Egdahl and Goldberg (3) insulin-like activity may be released from the liver after glucose infusion in anesthetized dogs Moreover Penhos et al (10) recently reported that immunoreactive insulin may be detected in the serum of anesthetized rats up to 72 hours after removal of all gastrointestinal and pancreatic tissue provided a functional liver was left in situ Eviscerated rats without a functional liver however maintained measurable serum insulin concentrations for 6 hours only

Circulating insulin appears to be taken up by the human myocardium above a certain insulin concentration level and released below that level (17) If an uptake/release relationship exists in the human liver an excess of circulating insulin secreted by a β-cell tumor may be bound to the liver and subsequently released into the bloodstream In this series a negative insulin concentration difference over the liver was observed only in the three insuloma patients who displayed hyperinsulinemia in response to β cell stimulation In the fourth patient with insuloma neither hyperinsulinemia nor an inverse relationship between portal and hepatic venous insulin concentrations could ever be induced

β-cell tumors often produce an increased amount of proinsulin (8 16) which appears to be less efficiently retained by the liver than insulin (12) Consequently a change in the relationship between insulin and proinsulin might be expected after passage of blood through the liver The immunoassay used in this investigation does not discriminate between insulin and proinsulin Therefore the differences between portal and hepatic venous IRI values can not be relied upon as absolute values However this fact cannot reasonably explain the inverse relationship between portal and hepatic venous IRI values found in this series

In conclusion this study has shown a higher insulin concentration in hepatic venous blood than in portal blood in three out of four patients with insuloma Whether this might be due to insulin secreting hepatic metastases too small to be detected by radiologic examination or to the release of insulin from the liver by some other mechanism remains to be resolved

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Diabetes Mellitus and Acute Myocardial Infarction

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ABSTRACT A series of 597 consecutive patients with acute myocardial infarction (AMI) have been screened for diabetes mellitus (DM) Six per cent of the series had DM which is exactly the frequency of DM in an age matched population This finding corresponds with results of other investigators, indicating that treated diabetics do not have an increased risk of AMI Diabetics suffering from AMI do not have an increased mortality nor do patients treated with oral antidiabetics have a higher mortality than those treated with insulin

It is generally accepted that patients with diabetes mellitus (DM) run a greater risk of ischaemic heart disease than non diabetics (12) During the last few years however the relationship between DM and acute myocardial infarction (AMI) has been discussed (8 19) Although the mortality from AMI is mainly reported to be higher in patients with DM than in non diabetics approximately 40–50% (13 15 18 19) some authors claim normal rates of death and complications in this group of patients (11 17) The importance of oral antidiabetics (OA) in AMI has been discussed since the report in 1971 of a high rate of complications in this group of diabetics (22)

This study was planned to investigate the frequency of DM in patients with AMI and to compare the rates of complications and mortality from AMI in diabetics especially in those treated with OA with those in non diabetic patients

MATERIAL

The material comprised 597 consecutive patients with AMI admitted to Bispebjerg Hospital Coronary Care Unit (CCU) in the period April 1 1974–July 1 1975 (comprising approximately 30% of the total number of hospitalized patients with AMI in the Copenhagen area in this period)

All patients were screened for DM (routine blood sugar (BS) and urine control) but only one patient had DM diagnosed during hospitalization The rest of the diabetics had been treated for several years controlled by a general practitioner or in hospitals A total of 37 diabetics was found Three patients were treated with diet but as BS and oral glucose tolerance test were normal they were not included in the series On admission 15 patients were being treated with insulin and 21 (plus the newly diagnosed patient) with OA The diagnostic criteria for AMI were those recommended by the WHO

All patients stayed in the CCU for approximately 14 days and ECGs were monitored for at least four days BS and urine were controlled daily

The following items were investigated the frequency of DM in the total material the fluctuations in BS and the treatment of the diabetics the frequency and type of arrhythmias the four week mortality and the cause of death

The frequency of previous infarctions has not been examined because population studies showed that old scars were present at autopsy in 45% of patients with no history of previous infarctions (14)

RESULTS

Among the 597 patients with AMI 37 (6 2%) had DM This group was further investigated Table I shows age of patients days of hospitalization duration of diabetes and the number of fatal cases in the DM group with a breakdown by type of diabetic treatment The general trend is for the patients treated with OA to be somewhat older and have a shorter history of DM The period of hospitalization and the mortality in the insulin and the OA group are the same

The frequency of arrhythmias in the CCU during the days of monitoring is shown in Table I The relationship between BS on arrival the frequency of arrhythmias and mortality and the causes of death are shown in Table II The majority of patients

Table I *Clinical data on the 37 diabetics*

	Insulin group	Oral anti diabetic group
No of pats	15	22
Mean age (y)*	66	72
Duration of hospitalization (d)	13	14
Duration of DM (y)	20	8
Mortality	5 (33 %)	6 (27 %)
Arrhythmias	5 (33 %)	12 (54 %)
Tachycardias	5	9
Bradycardias		5
Pulmonary oedema	3 (20 %)	5 (23 %)

* Average age of patients with AMI in this department 69 years

Table II *Arrhythmias and deaths in patients arriving with BS>12 mmol/l and causes of death within 4 weeks following AMI*

	Insulin group	Oral antidiabetic group
BS>12 mmol/l on arrival		
No of pats	10	10
Arrhythmias	3	10
Deaths	3	3
Causes of death		
Arrhythmias	1	
Shock	3	1
Other	1	5

Pneumonia 4 cerebral apoplexia 1

showed the highest BS on the first day of hospitalization

Fig. 1 shows the interval until death from the day of admission Fig 2 shows the interval from the first symptom to hospitalization compared with that for the total of AMI patients in this department according to the Danish Heart Registration Seventeen patients had been given sulfonyl urea drugs and five biguanides with a tendency to a higher mortality in the latter group No patients suffered from diabetic coma or severe hypoglycemia

DISCUSSION

The frequency of DM in our patients with AMI was 6 2% (4 4–8 4% with 95% confidence limits) a little lower than the range of 8–18% reported by other authors (5 9 10 18) The patients admitted to the CCU were in no way selected according to age or other disease Fig 2 shows that patients with and without DM were admitted with much the same interval from onset at all events the diabetics were not hospitalized later The expected frequency of DM in a population of the same age is 6% for Americans as well as Scandinavians (7 23) In a group of borderline diabetics treated with diet and OA Carlström (2) found the same frequency of AMI as in non-diabetics in contrast to a group of untreated borderline diabetics These findings and our observations although the number of patients is small seem to indicate that treated diabetics run no increased risk of myocardial infarction

The mortality from AMI in patients with DM is equal to the mortality in this department in the same period (33%) The OA treated patients showed the same mortality as the insulin treated although they were older (Table I)

The frequency of arrhythmias in the diabetics was the same as in the non-diabetics (40%) In this investigation bradycardia was more often seen in OA treated patients (Table I) whereas ventricular fibrillations in contrast to other reports were not observed (20)

On admission half of the patients treated with

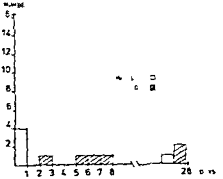


Fig 1 Time from admission to death in fatal cases

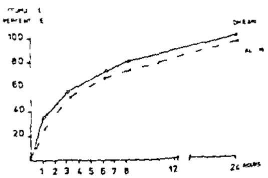


Fig 2 Time from start of symptoms to admission

OA and 2/3 of the insulin treated had high BS (>12 mmol/l). In the high BS group the OA treated patients had more arrhythmias than the insulin treated (Table II). In this material none of the patients arrived with hypoglycemia and this was not a precipitating factor. AMI is known to have a BS raising effect in non-diabetics but the influence on the clinical course of the infarction has not been investigated (4, 6). A conceivable explanation for the high BS is poor regulation of the diabetes but we have no reason to believe that this was so as all the patients were regularly controlled. The hyperglycemia may be caused by an increased sympathetic tone and a high concentration of noradrenaline in blood has been found in patients with AMI (3). Another explanation could be production of an insulin antagonist (12) or suppression of insulin secretion (1).

In our series approximately 1/4 of the patients treated with OA were discharged with a higher dose of OA or after having been switched to insulin. The treatment was unchanged in the insulin treated patients. A permanent increase in insulin demand after AMI has been found earlier (16, 21) but those patients were all treated with insulin and a number of them would today have received OA.

The frequency of cardiogenic shock tends to be higher in insulin treated patients as found by other authors (15, 16). The trend in patients in the present series who died was for the insulin group early deaths in cardiogenic shock, whereas in the OA group death occurred later and from intercurrent disease (Table II).

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Skeletal Lesions of the Feet in Diabetics and their Relationship to Cutaneous Erythema with or without Necrosis on the Feet

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ABSTRACT Seventy patients with cutaneous erythema of the feet with or without necrosis were the subjects of this investigation Sixty five of them had open diabetes The glucose tolerance of the remaining five patients was altered in a diabetic direction Twenty seven of the 70 patients had roentgenological ly demonstrable destruction in the bones of the feet These 70 patients were compared with 61 diabetic control patients of corresponding age and duration of diabetes but without these skin lesions of the feet Only four of the 61 control patients had destruction in the bones of the feet and all these destructions were small Precipitating factors were identified in general for the skin lesions, the most common being cardiac decompensation A higher frequency of precipitating factors was seen in patients with skeletal destructions than in those without The skeletal destructions and cutaneous necrosis are supposed to be equivalent le sions, localized to different tissues in the feet When patients presenting skin lesions of the feet in the form of distal gangrene were compared with those who had cutaneous erythema and necrosis of the feet but no distal gangrene, no differences were found with re spect to age duration of diabetes, occurrence of pre cipitating factors and the occurrence of skeletal de struction Cutaneous erythema without necrosis is understood to be incipient diabetic gangrene

An earlier paper (13) described areas of cutaneous erythema with or without necrosis localized to the legs and feet of diabetics Some patients reported in that paper also had destruction of the bones of the feet Cutaneous erythema or other skin lesions con comitantly *with Charcot joints in diabetics have* been described (8 17 20) In these reports the authors state that there are no signs of peripheral arterial insufficiency Destruction of the bones of the feet of diabetics is attributed by a number of authors to neuropathy possibly as a result of trau ma although there is no convincing evidence of this (13)

There is probably no clear distinction between cutaneous erythema with or without necrosis local ized to the legs and feet in diabetics and distal diabetic gangrene as stated previously (13) In most papers concerning diabetic gangrene (4 6 11 12) the gangrene was considered to be due to arterio- or *arteriolosclerotic occlusion of the arterial lumen* There is however no convincing evidence for this hypothesis (5 22) Goldenberg et al (10) called attention to what they described as proliferative changes in the endothelium of arterioles and consid ered these lesions to be the cause of diabetic gan grene Other authors (21 22) question whether they are of any special significance in the pathology of diabetic vascular disease and whether they are of an occlusive nature The importance of diabetic neuropathy for the development of diabetic gan grene is an open question as is the importance of diabetic microangiopathy The pathogenesis of dia betic gangrene is still not clear (5)

The purpose of this paper is to study more closely the possible relationship between cutaneous ery thema with or without necrosis localized to the feet and skeletal lesions of the bones of the feet in dia betics as well as the possible relationship between these skin lesions and distal diabetic gangrene

DEFINITIONS MATERIAL AND METHODS

Clinical part

In this report cutaneous erythema with or without necrosis (13) refers to lesions localized to the feet By arterial insufficiency is meant peripheral coldness and absence of pulses in both the dorsalis pedis artery and the

Table I. Data on 70 patients with cutaneous erythema with or without necrosis on the feet

	Not open diabetes	Open diabetes			
Age (y)	≥60	<60		≥60	
Duration of diabetes (y)		<10	≥10	<10	≥10
No. of pats	5	3	14	28	20
No. of women–men	2–3	1–2	8–6	12–16	12–8
Mean age (y)	78.2	54.3	43.4	71.9	71.0
Mean duration of diabetes (y)		4.7	19.7	4.2	17.3
Skin lesion					
Erythema	2	1	7	13	5
Erythema and necrosis	3	2	7	15	15
Precipitating factor to the skin lesion					
Cardiac decompensation with edema of the legs	5	0	0	10	8
Cardiac decompensation without edema of the legs	0	0	0	4	4
Nephropathy with edema of the legs	0	0	1	0	0
Arterial insufficiency	0	0	0	1 (3)*	0
Edema of the feet and/or legs of unknown cause	0	2	11	5	4
Unknown	0	1	2	8	4
Skeletal lesion of the foot					
Destruction, patients with concomitant demineralization included	1	1	6	8	11
Only demineralization	3	0	3	7	4
Neither destruction nor demineralization	1	2	5	13	5
Neuropathy	5	1	12	23	20
Atrophic circumscribed skin lesions (Melin)	1	1	7	14	13

* Two of these three patients also had cardiac decompensation

erior tibial artery. Distal gangrene refers to cutaneous necrosis of the toe(s) or heel.

Definitions of diabetes, neuropathy, edema, atrophic circumscribed skin lesions (18) and cardiac decompensation have been given earlier (13, 16). During the period 1969–74, 157 patients with cutaneous erythema with or without necrosis of the feet were examined clinically by one of us (F.L.) at the Department of Medicine. Roentgenological examinations were performed in 74 of these patients. The patients selected for roentgenological examination were generally those who had more severe skin lesions. Four diabetics were excluded from the material because they also had rheumatoid arthritis, primary gout or neoplasm. Of the remaining 70 patients, 65 had open diabetes. None of these patients had sarcoidosis or hyperparathyroidism and in no patient was a communication observed between cutaneous necrosis and the skeleton of the foot.

The significance of the difference between means was calculated using Fisher's *t*-test, and Fisher's exact probability test was used to test the difference between groups. $P<0.05$ was chosen as the level for statistical significance.

Roentgenological part

Roentgenological examinations were performed in the 70 patients with skin lesions of the feet and in 61 diabetic control patients of corresponding age and duration of diabetes. In all patients, the roentgenological examinations consisted of a frontal view, one or more oblique and often lateral views of the foot. Both feet were examined in 113 patients, only the right or the left foot in 18. The ankle joint was examined in four patients.

All X-rays were examined by one of us (S-O.H.). Roentgenologically demonstrable skeletal changes were classified without knowledge of the clinical findings. The degree of skeletal demineralization was estimated subjectively and the occurrence of diffuse or periarticular demineralization was recorded. The term destruction is used here to refer to the loss of skeleton from the phalanges, tarsals or metatarsals. New bone formation is defined as demonstrable periosteal bone production and thus not local or diffuse recalcification after earlier demineralization. Regression of skeletal lesions refers to total or partial reconstruction of bone destroyed earlier.

RESULTS

Clinical observations

As in earlier studies (13, 16), precipitating factors for the skin lesions could be established in most cases (Table I).

Table II *Data on 61 diabetic control patients without cutaneous erythema or necrosis on the feet*

Age (y)	<60	≥60	
Duration of diabetes (y)	≥10	<10	≥10
No of pats	13	34	14
No of women–men	5–8	18–16	6–8
Mean age (y)	45 9	72 3	70 6
Mean duration of diabetes (y)	28 5	5 3	18 4
Cardiac decompensation			
with edema of the legs	0	7	2
without edema of the legs	0	4	3
Nephropathy with edema of the legs	0	0	1
Arterial insufficiency	0	2	0
Skeletal lesion of the foot			
Destructions patients with concomitant demineralization included	0	4	0
Only demineralization	0	4	1
Neither destruction nor demineralization	13	26	13
Neuropathy	10	26	12
Atrophic circumscribed skin lesions (Melin)	11	19	9

The five patients who were not known to have diabetes were subjected to oral glucose tolerance tests and the results were treated as described earlier (13) The glucose tolerance of these five patients was impaired in comparison with that of the controls (mean±S E M for patients 614±50 5 for controls 456±16 5) The difference between the means for the two groups yielded $t=3\ 27$ d f =38 $p<0\ 005$

As is evident from Tables I and II the occurrence of destruction of the bones of the feet is more common in patients with skin lesions of the feet (27/70) than in patients without such skin lesions (4/61) ($p<0\ 001$) The occurrence of factors known to precipitate the skin lesions of the feet was more common in patients with destruction of the bones of the feet (25/27) than in patients without such destruction (30/43) ($p=0\ 018$) Destruction or demineralization of the bones of the feet is more common in patients with erythema and necrosis of the feet (29/41) than in those with erythema alone (15/29) ($p=0\ 05$) Patients with skin lesions and destruction of the bones of the feet were older and had diabetes of longer duration (mean 68 and 12 years respectively) than patients with skin lesions but without either destruction or demineralization of the bones of the feet (mean 64 and 10 years respectively) The differences are however not significant

There was no significant difference in the occurrence of neuropathy in patients with skin lesions of the feet compared with control patients 61/70 and 48/61 respectively ($p=0\ 08$) nor was there any difference in the occurrence of neuropathy in patients with skin lesions and skeletal destruction (25/27) compared with patients with skin lesions but no skeletal destruction (36/43) ($p=0\ 17$) There was no significant difference with respect to age or duration of diabetes between patients with cutaneous erythema and necrosis in the form of distal gangrene (mean 68 and 10 years respectively) and patients with cutaneous erythema and necrosis of the feet but no distal gangrene (mean 69 and 9 years respectively) In the latter patients there were no significant differences concerning the occurrence of precipitating factors to the skin lesions (21/26 and 13/15 respectively $p=0\ 31$) or the occurrence of skeletal destruction (13/26 and 5/15 respectively $p=0\ 15$)

Eleven of the 27 patients with skin lesions on the feet and skeletal destruction had cutaneous erythema with necrosis in the region of the skeletal destruction and five had previously had cutaneous erythema with necrosis in the vicinity of the skeletal destruction Neuropathy was demonstrated in all patients with destruction of the bones of the feet with the exception of two in whom pronounced edema made it impossible to ascertain whether or not neuropathy was present Three patients with arterial insufficiency and skin lesions had either destruction or demineralization of the bones of the feet

The skin lesions healed in 63 of the 70 patients Three patients died before the skin lesions could heal Two patients underwent amputation of a lower leg and two of a toe Regression of skeletal destruction was seen in five of 14 investigated patients after

Table III *Roentgenologically demonstrated lesions of the bones of the feet in 44 of 70 patients with skin lesions of the feet*

	Not open diabetes	Open diabetes			
	≥60	<60		≥60	
		<10	≥10	<10	≥10
Age (y)					
Duration of diabetes (y)					
No of pats	4	1	9	15	15
No of women–men	2–2	1–0	5–4	8–7	10–5
Skeletal lesions of the feet	4	1	7	14	11
Demineralization	0	0	2	1	3
Periosteal new bone formation					
Destruction patients with concomitant demineralization included	1	1	6	8	11
Regression of destruction	0	0	2	1	2

14 patients investigated

immobilization or after treatment of the cardiac decompensation and local treatment consisting in elimination of edema of the lower legs and feet Seven patients had progressive skeletal destruction of the bones of the feet and two had unaltered destruction None of these seven patients received the treatment mentioned only one of the latter two received the same treatment as the above mentioned five patients with regression of skeletal destruction

None of the control patients with skeletal destruction of the bones of the feet (Table II) had had cutaneous necrosis earlier in the vicinity of this destruction but two of them had had bilateral edema of the legs for many years

Roentgenological observations

Skeletal lesions were demonstrated in 44 of the 70 patients with skin lesions of the feet (Table III) The demineralization in the bones of the feet was often of a patchy nature In 27 of the patients with skin lesions on the feet there was destruction of the bones of the feet The localization of the destruction is given in Table IV In seven patients destruction was observed to the bones of both feet The joint surfaces were often preserved even though many cases showed pronounced periarticular destruction In other patients there was articular destruction with skeletal fragmentation Five patients had a pronounced loss of skeleton in the metatarsals leaving only small parts of the bases of these As will be seen from Table IV the distal phalanges were involved in 10 patients and most often the mineral had totally disappeared from these Regression of skeletal destruction was observed in five patients two of whom showed almost complete reconstruction of previously destroyed bones (Fig 1)

Table IV *Sites of involvement of skeletal destruction in 27 patients*

	Not open diabetes	Open diabetes			
	≥60	<60		≥60	
		<10	≥10	<10	≥10
Age (y)					
Duration of diabetes (y)					
No of pats	1	1	6	8	11
No of women–men	0–1	1–0	4–2	2–6	6–5
Localization of the destruction					
Distal phalange	0	0	2	4	4
Distal interphalangeal joint	0	0	0	0	3
Proximal interphalangeal joint	0	0	0	1	4
Metatarsophalangeal joint	1	1	3	1	7
Tarsometatarsal joint	0	0	1	0	1
Tarsal joint	0	0	0	0	3
Metatarsal	0	1	5	3	4
Ankle joint	0	0	0	1	0

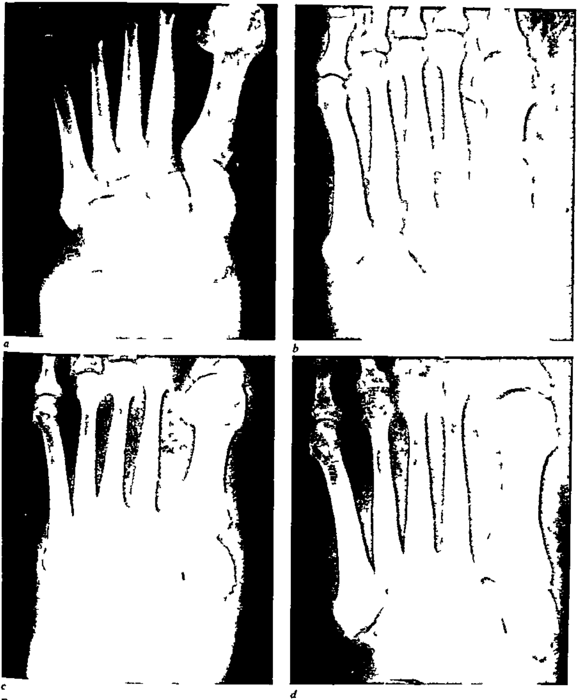


Fig 1 Female 56 years old d abetes of 15 years dura tion Edema and cutaneous erythema of the nght lower leg and foot for three months previous to the first X ray There was no cutaneous necrosis in her med cal history Small skeletal destruct on of the base of the second meta tarsal and first tarsometatarsal jo nt (*a*) Four months later (*b*) the destruction had progressed to include the base of the first metatarsal where a great number of skeletal fragments were seen The bases of the second third and fourth metatarsals were destroyed or deformed as were the tarsometatarsal joints of the same toes One year after the first X-ray there was an obvious rest tut on of the skeletal structure (*c*) The fragmentation had disappeared but there was still a pronounced deformation of the tarsometatarsal joints Two years and three months after the first examinat on still more regression was observed and the deformat on was now moderate (*d*)

Four of the 61 control patients (Table II) had destruction of the bones of the feet All these latter destructions were small compared with those generally seen in the above mentioned patients with skin lesions of the feet

Skeletal destruction was sometimes observed to develop rapidly In two of the patients with skin lesions pronounced progression was registered at intervals of only 23 and 28 days respectively

Periosteal new bone formation was observed in six patients (Table IV) in two of them in connection with regression of skeletal destruction Seventeen of the patients with skin lesions on the feet 13 of them women and five of the controls three of them women had only demineralization of the bones of the feet Twenty of the 27 patients with skin lesions on the feet and concomitant roentgenologically demonstrated skeletal destruction also had demineralization of the bones of the feet

DISCUSSION

As reported earlier (13 16) certain factors most often cardiac decompensation can precipitate cutaneous erythema with or without necrosis or purpura on the lower extremities in diabetics It was maintained that these lesions were due to an altered reaction in certain diabetics It was suggested that this altered reaction is due to an impaired transport of oxygen from the blood in the capillaries to the cells of the tissue and/or transport of metabolites from the tissue due to diabetic microangiopathy and possibly neuropathy The presence of peripheral neuropathy in diabetics impairs the capacity to vary the blood circulation to the skin area in question (9 19) It is not clear why there is sometimes cutaneous erythema with or without necrosis and sometimes only purpura It is worth noting however that cutaneous erythema is often accompanied by purpuric lesions within the area of erythema This altered mode of reaction was also demonstrated directly by comparing the reactions of diabetics and non diabetics to local thermal trauma (14)

Other authors (1 3) have also suggested that diabetic microangiopathy could make diffusion through the walls of the capillaries more difficult An impaired diffusion has not been demonstrated experimentally (22) but the experimental conditions were not satisfactory

Precipitating factors were identified in general for the erythema with or without necrosis seen in this study Destruction of the bones of the feet was more common in patients with these skin lesions than in control patients Precipitating factors in patients with skin lesions on the feet were identified more often when skeletal destruction in the feet was present as well

The time of the appearance of these skin lesions—within the course of a few days to a few weeks—was known in most cases The erythema disappeared most often after one or a few weeks The necrosis healed much more slowly The connection in time between precipitating factors and the development and disappearance of the skin lesions described was usually apparent There are obvious reasons why the time of onset of the skeletal destruction could not be determined This destruction seems to develop within the course of a few weeks while its regression probably occurred much more slowly The connection between the skin lesions and skeletal lesions especially the fact that precipitating factors for the skin lesions were more common in patients with skeletal destruction than in those without speaks in favour of the skeletal lesions as well as the skin lesions being precipitated by certain well defined factors It is plausible to suppose that the skin necrosis and the skeletal destruction are equivalent lesions localized to different tissues in the feet

Patients with familial amyloidosis and polyneuropathy have been found to have similar skeletal destruction to diabetics (15) In the case of the patients with amyloidosis there is no evidence of occlusive vascular disease (2 15) Skeletal destruction in the feet of diabetics has been attributed earlier to neuropathy In the present study no obvious difference in the occurrence of polyneuropathy was found between diabetics who had cutaneous erythema with or without necrosis and those who did not or between patients with both skin lesions and skeletal destruction and those with skin lesions alone These findings run counter to the assumption that polyneuropathy is of decisive importance in the pathogenesis of skeletal destruction in the feet of diabetics

Only demineralization of the bones of the feet was observed both in patients with skin lesions and in those without However demineralization was more common in the former group Demineralization was also more common in women than in men which may possibly be due to a higher frequency of osteoporosis in older women than in men

In an earlier paper (13) it was stated that there probably is no clear distinction between cutaneous erythema with or without necrosis localized to the legs and feet in diabetics and distal diabetic gangrene Diabetic gangrene is generally used to refer to distal gangrene For that reason it was of interest to compare patients who have skin lesions in the form of distal gangrene with those presenting cutaneous erythema with necrosis but not gangrene No differences were found with respect to age duration of diabetes occurrence of precipitating factors such as cardiac decompensation and the occurrence of skeletal destruction The conception that cutaneous erythema with or without necrosis and distal gangrene have the same cause is supported by these results It is probably more correct to refer to cutaneous erythema with necrosis as diabetic gangrene and to cutaneous erythema without necrosis as incipient diabetic gangrene For historical reasons however we have chosen to use terms such as distal gangrene and cutaneous erythema with or without necrosis Diabetic gangrene has been reported earlier to be localized not only to the acra of the lower extremities but also to the dorsa of the foot and sometimes appears as patchy areas on the lower leg (7 10 11)

Cutaneous erythema localized to the legs and feet has not been described earlier as incipient cutaneous gangrene neither has cutaneous erythema with or without necrosis been described as generally being precipitated by certain demonstrable factors such as cardiac decompensation Erythema surrounding diabetic cutaneous necrosis on the lower extremities has hardly been reported

ACKNOWLEDGEMENT

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Body Composition and Glucose Metabolism in Hypertensive Middle-aged Males

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ABSTRACT Body fat, body cell mass, fasting blood sugar, glucose tolerance and fasting insulin have been determined in 106 hypertensive males aged 47–54 years and in 41 normotensive 50 year old males Both groups were derived from screening examinations in random population samples The hypertensive subjects were more often obese and had more often an impaired glucose tolerance and a higher fasting insulin compared with the normotensive subjects The metabolic differences were not explained simply by the higher degree of obesity in the hypertensive subjects as the differences remained when the hypertensive subjects were matched for body fat with normotensive controls The impaired glucose metabolism demonstrated quantitatively in an unselected group of hypertensive subjects might be one of the factors explaining the variable prognosis in hypertensive subjects

The natural course of untreated essential hypertension is extremely varied some subjects surviving to high age without complications while others are struck by myocardial infarction and stroke and die at a young age (8) Prognostic factors other than the blood pressure are therefore needed to define the indications for treatment Some prognostically unfavourable factors such as eyeground changes (13) and heart and kidney involvement (22) are known but cannot explain the poorer prognosis in hypertensives without such changes compared with normotensives (11) Overweight and obesity curtail the expectation of life among hypertensives (4) but the pathophysiological mechanism behind this finding has not been clarified Obesity has been shown to be associated with impaired glucose metabolism (21) which in some studies has been related to increased risk of coronary and cerebral atherosclerotic disease (23) This indicates a possible explanation of the susceptibility of the obese hypertensives Impaired glucose metabolism has been related to increased prevalence of hypertension and the finding was not attributable simply to overweight (23) However quantitative data are lacking on glucose metabolism in groups of normo- and hypertensive subjects homogeneous with respect to age and sex

The aim of the present investigation was to study glucose metabolism in normo and hypertensive middle aged males Body fat (BF) and body cell mass (BCM) were determined in order to analyse whether possible differences in glucose metabolism could be explained simply on the basis of differences in body composition

POPULATION SERIES AND METHODS

One hundred and six previously untreated male hypertensives were recruited from a screening examination of a random population sample (25) The participation rate was 75% Subjects with BP above 170 mmHg systolic and 105 diastolic on two occasions two weeks apart were included in the hypertension group All had benign essential hypertension as judged by a negative examination for secondary hypertension (24) Serum creatinine test for albuminuria and isotope renograms were normal in all subjects Their ages ranged from 47 to 54 years (average 50 ±3 4) Within this range age was not significantly related to any of the variables studied except for a weak positive correlation with fasting blood sugar ($r=0\ 27$)

A reference group of 58 subjects was recruited from another screening examination in a random 3% subsample of all 50-year-old males in Goteborg All had BP below

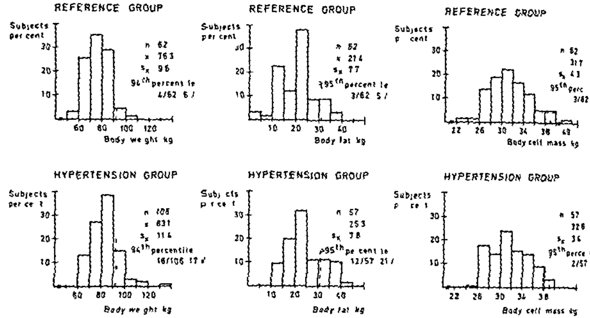


Fig 1 Distributions of body weight body fat and body cell mass

170 systolic and 105 mmHg diastolic The participation rate was 77 %

The informed consent of all participants was obtained after the nature of the procedures had been fully explained All participants had normal serum potassium values and there was no difference between normotensives and hypertensives An oral glucose tolerance test and determination of fasting insulin were done in 41 consecutive cases of the 58 normotensives and in all hypertensives

BP was measured in the seated position after about min rest It was recorded with a rubber cuff 12 5 cm ide and 26 cm long connected to a mercury manometer Diastolic BP phase 5 i e when the sound disappears was used The weight was measured by a lever balance to the nearest 0 5 kg

The total body potassium content was determined in a randomly selected half ($n=57$) of the hypertension group and in the total reference group using a whole body counter (20)

BF was calculated according to the formula by Forbes et al (7) body weight $-\dfrac{\text{total body potassium}\times 14\ 68}{1\,000}$

BCM was calculated as $\dfrac{\text{total body potassium}\times 8\ 33}{1\,000}$

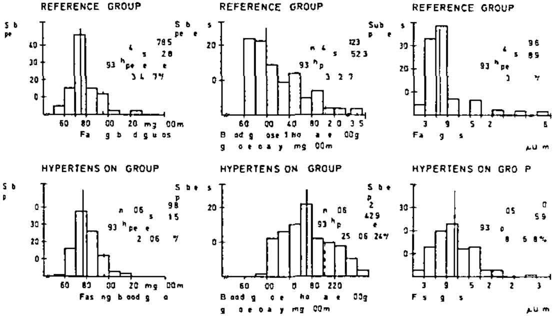
according to Moore et al (15)

The blood glucose concentration was determined by means of a glucose oxidase method using a commercially available reagent (Glox® Kabi Stockholm Sweden) and with glycerin buffered perchloric acid as protein precipitating agent The serum insulin concentration was determined by a double antibody method using a commercial radioimmunoassay kit (Phadebas Pharmacia Uppsala Sweden)

The oral glucose tolerance test was performed at 8 a.m The subjects were told not to change their normal dietary habits but to avoid fasting except for the overnight fast prior to the oral glucose tolerance test not to smoke and to avoid physical exercise that morning The participants were seated during the test and venous blood samples were drawn in the seated position for fasting blood sugar and insulin determinations Thereafter 100 g glucose was given orally The blood sugar concentration was again determined after 60 min In the reference group and in a subsample of the hypertension group ($n=14$) the blood sugar concentration was also determined 30 90 and 120 min after the glucose load Of these 4 determinations the blood sugar concentration after 60 min had the highest correlation coefficient $r=0\ 95$ to the sum of the 4 blood glucose determinations

The participants classified their degree of physical activity at work and during leisure time according to a 4-point scale (17) The validity of the scale has been tested against objective methods (9)

The primary objective of the present study was to compare hypertensive men with normotensive men It soon turned out however that there were differences in body composition between the two groups which might confound the findings concerning glucose metabolism To avoid any bias homogeneous blocks with regard to BF and BCM were constructed All hypertensive and normotensive individuals were filled out on a scatter diagram with BF and BCM on the axes The values of the other variables were not known at this moment Thirty two of the 41 men in the reference group were suitable for this blocking No proper controls for the most obese hypertensive men could be found in the random subsample of normotensive men All obese men of that population study had however been studied and 6 of these obese normotensive men were added in suitable blocks In spite of this one extremely obese hyperten-



F g 2 Distribut ons of fast ng blood sugar glucose tolerance and fast ng nsul n

sive man d d not f t nto any block and he was there fore excluded from th s part of the analys s Thus 38 normo ens ve men were available n the blocks and compared w h 56 hypertens ve men

The block l mits were chosen only on the basis of BF and BCM For BF the block l m ts were 11 0–?0 9 ?1 0–6 0 and 26 1–44 0 kg The d str but on of BCM d d not d ffer between the three BF blocks Thus only the block ng according to BF was necessary

Means standard dev at ons and correlat on coeff c ents were calculated acco d ng to standard methods D f feren es in means between two groups were tested us ng S udent s t test The blood sugar concentrat on 1 hour after oral glucose load ng and fast ng nsul n concentrat on were transformed logarithm cally as these variables we e d stributed skewedly (F g 2) D fferences n proport ons were tested us ng the χ^2 test Only ? s ded tests were used The accepted level of stat stical s gn f cance was $p<0.05$

RESULTS

Body compos t on

The d stribut ons and means of body we ght body fat and body cell mass in the reference and hyper tension g oups are sho vn in Fig 1 Body weight was s gn ficantly h gher in the hypertens on group This difference was mainly expla ned by a h gher BF in the hypertensives while there was no d f ference in BCM The 95th percentile in the ref

Table I *Son e l near correlat on coeff cients (r) and the level of stat st cal s gn f cance (p)*

BS 60 =blood sugar 60 min after 100 g glucose orally

	Reference group			Hypertens on group		
	n	*r*	*p*	*n*	*r*	*p*
We ght–body fat	58	0 65	0 001	57	0 8?	0 001
We gh –body cell mass	58	0 6?	0 001	57	0 68	0 001
Body fat–body cell mass	?8	0 ?0	n s	57	0 14	n s
Fas ng blood sugar BS 60	41	0 4?	0 01	106	0 5?	0 001
Fas ng blood sugar fast ng nsul n	41	0 ?6	0 001	106	0 ?1	0 05
Fast ng nsulin BS 60	41	0 18	n s	106	0 11	n s
Body fat fasting blood sugar	41	0 43	0 01	57	0 13	n s
Body fat BS 60	41	0 30	n s	57	0 03	n s
Body fat fast ng nsul n	41	0 43	0 01	51	0 ?7	0 01

Table II *Glucose and insulin in three groups of hypertensive subjects and normotensive controls blocked according to body fat*

BS 60 = blood sugar 60 min after 100 g glucose orally

	Normotensives			Hypertensives		
	n	*x*	s_x	*n*	*x*	s_x
Body fat 11 0–20 9 kg						
Fasting blood sugar	11	76	12 4	16	80	7 8
BS 60	11	110	46 6	16	171	45 4
Fasting insulin	11	6 5	2 5	16	12 1	6 7
Body fat	11	16 6	3 3	16	16 9	3 2
Body cell mass	11	30 5	3 6	16	33 3	3 4
Body fat 21 0–26 0 kg						
Fasting blood sugar	12	80	8 3	19	84	11 5
BS 60	12	138	70 5	19	187	49 5
Fasting insulin	12	7 5	3 0	19	10 5	5 4
Body fat	12	23 3	1 3	19	22 9	1 4
Body cell mass	12	31 4	3 8	19	31 9	4 3
Body fat 26 1–44 0 kg						
Fasting blood sugar	15	87	16 7	21	80	14 8
BS 60	15	144	32 3	21	167	37 2
Fasting insulin	14	17 9	14 0	21	12 4	6 9
Body fat	15	33 4	3 4	21	32 8	4 9
Body cell mass	15	31 7	2 9	21	32 3	3 3

erence group was calculated and used for assessing the proportion of "abnormal" values in the hypertension group A significantly higher proportion of body weight and BF above the 95th percentile was found in the hypertension group indicating a higher percentage of obese subjects in this group

Positive correlations of about the same strength in both groups were found between body weight and BF and between body weight and BCM (Table I)

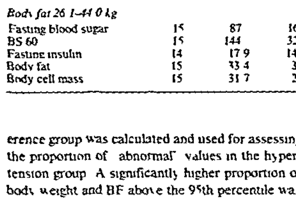


Fig 3 Relationship between systolic blood pressure and glucose tolerance ●=hypertensives O=normotensives

Glucose metabolism

The distributions for fasting insulin concentrations and for blood sugar concentration 60 min after oral glucose loading were skewed to the right (Fig 2) There were no differences either in mean fasting blood sugar between the two groups in distribution or in proportion of subjects above the 95th percentile The blood glucose after oral glucose loading and the fasting insulin concentration were significantly higher in the hypertension group and there was a shift of the median and mode to the right in the hypertension group

Fasting blood sugar and blood sugar 60 min after 100 g oral glucose were positively correlated with each other in both groups (Table I) Fasting blood sugar was positively correlated with fasting insulin All participants had normal serum potassium values and there was no difference between normotensives and hypertensives

The relationship between blood pressure and glucose metabolism

There was a weak but significant positive linear correlation between systolic BP and blood glucose concentration 1 hour after oral glucose loading when the two groups of men were combined

(Fig 3) No significant correlations were found within either group separately There was no significant linear correlation between systolic BP and fasting blood glucose or fasting insulin when the reference and hypertension groups were combined

Physical activity and glucose metabolism

There was no difference in fasting blood sugar blood sugar after oral glucose loading or fasting insulin between groups of hypertensives with different levels of physical activity during work and leisure time divided according to the 4 point scale

The relationship between body composition and glucose metabolism

BCM was significantly and negatively correlated with blood glucose concentration after glucose loading both in the hypertension group ($r=-0\ 45$) and in the reference group ($r=-0\ 27$)

Fasting blood sugar insulin concentrations and blood sugar 60 min after oral glucose loading in hypertensive men and normotensive men divided in blocks (see Methods) with regard to BF are given in Table II

Among the normotensive men the blood sugar concentration 60 min after oral glucose loading and fasting insulin increased significantly with increasing BF There was also a tendency towards higher fasting blood sugar with increasing BF but this trend was not statistically significant The impairment of glucose metabolism with increasing obesity was also obvious from the linear correlation coefficients in the reference group (Table I) between BF and the glucose metabolism variables

In the group with the lowest BF (11 0–20 9 kg) the hypertensives had significantly higher blood sugar concentration after oral glucose loading and higher fasting insulin than the normotensives The fasting blood sugar concentration was somewhat higher in the hypertensives but the difference was not significant In the groups with intermediate BF (21 0–26 0 kg) the hypertensives had significantly higher blood sugar concentration after oral glucose loading than the normotensives while there were no significant differences in fasting blood sugar or insulin concentration In the groups with the highest BF (26 0–44 0 kg) no significant differences were found between the hypertensives and the normotensives

DISCUSSION

The subjects in the reference group were chosen *at random* from the total population of 50 year old men i Goteborg The subjects with untreated hypertension were also recruited from a general population sample but one subject with extremely high BP was excluded as antihypertensive treatment had already been initiated As most of the variables studied are age and sex-dependent we chose to study hypertensive men within a narrow age span

The investigation of glucose metabolism was performed at the same time of day and during the same time of the year in both groups The reported diurnal and seasonal variations in glucose metabolism (3) have therefore been ruled out

The oral glucose tolerance test was performed with a fixed dose of 100 g glucose i e the dose was not calculated with regard to body weight A negative correlation was found between BCM and blood glucose after glucose loading in both groups which might be explained by this fixed glucose dose As there was no difference in BCM between the reference group and the hypertension group it is improbable that the relationship found would distort the conclusions regarding differences between normo- and hypertensive subjects

Our finding that hypertensives were overweight and that this was mainly caused by increased BF has previously been well documented as reviewed by Chiang et al (4) Previous studies (5 10 23) have indicated an impaired glucose metabolism in men with mild to moderately severe hypertension as was also clearly shown in the present study The impaired glucose metabolism in the hypertensive subjects was not explained by the higher degree of obesity in the hypertensives This is in agreement with the results reported by Stamler (23) but in contrast to the finding of Hedstrand (10) who observed no difference in glucose tolerance but somewhat higher fasting insulin concentration in hypertensive middle aged men compared with weight matched normotensive controls

One can only speculate about the possible mechanisms behind our findings It is impossible to state whether the tendency towards high BP or towards disturbed glucose metabolism was the primary disturbance or whether the association between the two is genetically mediated via some underlying factor In the hypertensive group antihypertensive treatment was shown to glucose tolerance (1) Although this

that hypertension is the primary disturbance the antihypertensive treatment might also directly influence the glucose metabolism which makes conclusions hazardous

Studies of regional haemodynamics have shown that hypertensives have a higher muscle blood flow and a lower arteriovenous difference for oxygen than normotensive subjects (2) This finding might indicate an arteriovenous shunting in the vascular bed of the muscles of the hypertensive subjects resulting in a smaller glucose entry into the extracellular fluid and into the muscle cells This haemodynamic setting in the hypertensive subjects is accordingly a possible explanation of the higher blood glucose concentration one hour after an oral glucose load in the hypertensives compared with the normotensives

The above suggestions cannot however account for the higher fasting insulin concentration in the hypertensive men The chronic insulin release has been shown to be stimulated by β receptor mediated sympathetic activity (16) which in turn has been found to be higher in hypertensive than in normotensive subjects (14) The peripheral uptake of glucose has been suggested to decrease with increase in β receptor mediated sympathetic activity (18) resulting in decreased insulin sensitivity which leads secondarily to an increase in insulin release Increased sympathetic activity would therefore theoretically lead to an increase in the chronic insulin release via both these mechanisms [illegible]c nervous activity has not been determined in the present study but a possible explanation of the higher fasting insulin in the hypertensives might be a higher than normal sympathetic discharge In animal studies an increased sympathetic discharge and an increased muscle flow have indeed been shown to occur as part of the so-called alarm reaction and has been suggested to play a role in the development of essential hypertension (6)

Hyperinsulinaemia (12) and decreased glucose tolerance (21) are frequently found in obesity These metabolic disturbances were also found in the hypertension group The hypertensive subjects had these disturbances even in the absence of obesity suggesting a different explanation in obesity and hypertension Increased sympathetic nervous activity might however be a common mechanism for the impaired glucose metabolism and the haemodynamic changes in early essential hypertension

The impaired glucose tolerance in roughly one fourth of the hypertensive subjects is probably associated with an increased risk of coronary heart disease and cerebrovascular disease as shown previously (23) Impaired glucose tolerance might thus be one of the factors related to the very variable prognosis in essential hypertension (8) and if present call for close supervision of both BP and possible further deterioration of the glucose metabolism to overt diabetes

The prognostic implications of an impaired glucose metabolism in hypertensive subjects must however be studied prospectively In the light of the great need for other prognostic factors apart from BP as a tool for therapeutic guidance (8) this type of study should be given high priority

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Hereditary Hepatic Porphyrias in Finland

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ABSTRACT The occurrence of hepatic porphyrias —acute intermittent porphyria (AIP) and variegate porphyria (VP)—in Finland has been studied During a period of 9 years 107 patients with AIP and 45 patients with VP were found The prevalence of hereditary hepatic porphyrias was calculated to be 3 4 per 100 000 inhabitants The patients belonged to 42 different families Eighty nine patients (59%) had had acute attacks, whereas 63 were symptomless latent cases Precipitating factors symptoms and excretion of porphyrins and their precursors did not significantly differ from what has been reported earlier from other parts of the world A slight fragility of the skin on the back of the hands was noted in some 50% of VP patients Abnormal sensitivity to sunlight could not be seen in a single case However about 50% of patients with VP showed an abnormal reaction when irradiated with artificial ultraviolet light The difference in the skin symptoms in South African and Finnish VP patients is discussed

Hereditary hepatic porphyrias are divided into three types each of them probably represents a separate disease caused by a specific mutation Acute intermittent porphyria (AIP) is the most usual type Since Waldenstrom s classical work (50) it has often been called the Swedish type of porphyria In Sweden there are more than 600 documented patients suffering from AIP (51 55) Numerous cases have also been reported from Denmark (57) from other parts of Europe (3 6 11 12 17) from the United States (29 43 53) and from Australia (32) The acute porphyric attack common to all hereditary hepatic porphyrias has been well documented in these reports

Variegate porphyria (VP) which is associated with skin symptoms is common in South Africa (7 9) but relatively few patients have been reported from other parts of the world (15 18 46 48 58) Hereditary coproporphyria is evidently the rarest type of hereditary hepatic porphyrias only a few dozen cases having been published (14)

A few cases of porphyria have been reported from Finland Since the first report in 1928 (26) a dozen reports quoted by us earlier (34) have been published mainly in Finnish periodicals In total some 30 cases of acute porphyria have been described in these papers An accurate classification of most of these cases is impossible however because of the scanty biochemical data given

The purpose of the present communication is to report clinical biochemical and genetic data on hereditary hepatic porphyrias in Finland

PATIENTS

Patients with acute porphyria have been systematically collected from the University Central Hospitals in Helsinki and Turku from the Provincial Central Hospitals and occasionally from District Hospitals All relatives of the porphyric patients who could be reached were studied in order to detect latent cases Fifty seven patients were found in different hospitals and 78 were diagnosed during family studies

The diagnostic criteria used were as follows AIP clearly elevated urinary porphobilinogen (PBG) excretion in acute and latent stages together with normal or slightly elevated faecal porphyrins VP clearly elevated faecal porphyrins especially protoporphyrin together with normal or slightly elevated urinary PBG excretion in the latent stage In seven cases belonging to biochemically verified porphyric families the post mortem diagnosis was based on the typical symptoms documented in hospital case histories We found 17 cases published earlier (27 40 41 54 59) to be members of the families studied by us They were therefore included in the clinical part of the present study In three cases with normal biochemical findings the diagnosis of porphyria was made indirectly by taking into consideration the Mendelian dominant inheritance Verified porphyria was found in

Table I *Type of porphyria occurrence of acute symptoms and sex distribution of 152 patients with hereditary hepatic porphyria*
Figures within parentheses=percentage

	Patients with acute attack			Symptomless patients			All patients		
	Total	Males	Females	Total	Males	Females	Total	Males	Females
Acute intermittent porphyria	73	21 (29)	52 (71)	34	18 (53)	16 (47)	107	40 (37)	67 (63)
Variegate porphyria	16	2 (13)	14 (87)	29	17 (59)	12 (41)	45	19 (4?)	26 (58)

their children and either in one of their parents or in a sibling

Ancestors have been traced in every family up to the 19th and in some cases up to the 17th century At the turn of 1974 the patients were contacted personally or by letter in order to clarify whether they had had symptoms due to porphyria after the diagnosis of porphyria had been made and information about precipitating factors was given *Ninety five (85%) out of 112 patients were reached*

The following terms are used to distinguish the different stages of porphyria irrespective of type acute porphyria for porphyria with acute symptoms latent porphyria for porphyric subjects symptomless at the time of examination whether or not they had had symptoms

METHODS

Laboratory methods The quantitative analyses of porphyrins and their precursors were made from mixed 24 hour urine Sodium carbonate was added in the collecting bottles in order to keep the urine alkaline Urinary δ aminolaevulinic acid and PBG were determined according to Mauzerall and Granick (31) urinary uro- and copro-porphyrin and faecal copro- and protoporphyrin according to Rimington (38) Coproporphyrin isomer distribution was measured according to Jensen (21) as modified by Koskelo and Toivonen (24) Free erythrocyte protoporphyrin was determined as described by Koskelo (22) Quantitative plasma hemopexin determinations were made by means of immunoelectrophoresis technique (28)

Light sensitivity tests were performed in 14 patients with VP and in 16 control subjects using a method outlined by Runge and Watson (39) The abdominal skin was radiated with a commercially available combined ultra violet and infrared lamp (Höhensonne 100 Original Hanau Fed Rep Germany) for 40 min at a distance of 23 cm Under these conditions the measured irradiance of the mercury UV lamp in the Soret region (404 5–405 0 nm) was 530 μW/cm² A 1 mm thick Schott WG 3 filter which filters 95% of wave lengths below 370 nm was placed between skin and lamp During the test the temperature at the skin was kept between 41 and 42 C

In the test all the control subjects had temporary erythema (evidently due to temperature) which subsided within 30 min This was regarded as a normal reaction The light test was judged as abnormal if a distinct erythema occurred about an hour after finishing the radiation

RESULTS

Prevalence

According to the criteria used, the diagnosis of hereditary hepatic porphyria was made in 15? cases 107 AIP and 45 VP (Table I) At the beginning of 1975 120 patients—83 AIP and 37 VP—were still alive In the light of these figures the prevalence of AIP in Finland is 2 4 of VP 1 0 and of hereditary hepatic porphyrias altogether 3 4 per 100 000 inhabitants above the age of 15 years

Genetic and geographical aspects

The AIP patients belonged to 35 families and the VP patients to 7 families The number of porphyric subjects in these families varied from 1 to 12 Porphyria was diagnosed in two or more successive generations in 20 families with AIP and in 6 families with VP In the families with 10 AIP patients no other cases with porphyria could be found No relatives could be reached in 4 families In 6 families all living members including both parents in four had the urinary and faecal porphyrin output within the normal range In AIP families 81 (40%) out of 207 first degree relatives (over 15 years) investigated were porphyric against 39 (48%) of 82 in VP families An incomplete penetrance was additionally verified by demonstration of a generation skip in three instances (see section Patients)

Two patients with AIP were so-called Skolt Lapps a very small ethnic group of a few hundred persons living in North Finland the other patients were Finns The geographical distribution of the origin of 32 porphyric families is presented in Fig 1 There is only one family originating from North Finland It may be noted that the population in

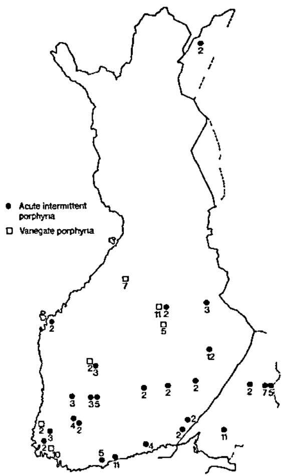


Fig 1 Places of birth of the oldest known carriers of the porphyric gene in 25 families with acute intermittent porphyria and in 6 with variegate porphyria The figures show the number of patients related to each family The broken line shows areas which belonged to Finland before the second world war

North Finland is sparse and the information which we received from the two central hospitals in that area was incomplete

Precipitating factors

Out of 89 patients with symptoms 13 were known to have taken barbiturates before the onset of abdominal symptoms Out of 48 patients with neurological symptoms at least 34 had received barbiturates in hospital during the abdominal symptoms but before the appearance of pareses It may be stressed however that 16 patients with abdominal symptoms had received barbiturates during their illness without any resulting neurological symptoms There are also at least five latent porphyric subjects who had received barbiturates during anaesthesia without provocation of symptoms

Of other well known precipitating factors the following were present before the onset of abdominal symptoms infection in 9 cases sulphonamides in 4 alcohol in 3 glutethimide meprobamate fasting menstruation and extraction of a tooth in one case each A fern extract preparation was apparently the precipitating factor in one fatal case Two patients developed abdominal symptoms after painting a house

The symptoms appeared in three patients during pregnancy and in another three about two months after delivery On the other hand 46 porphyric women in our material had had more than 100 full term pregnancies without provocation of porphyric symptoms

The acute attack

The occurrence of acute attacks and the sex distribution of the patients are presented in Table I About 75% of patients with symptoms were females The age at appearance of the first symptoms is presented in Fig 2 More than 90% of the patients had symptoms before the age of 40 the youngest patient being 13 There was no difference in age distribution between AIP and VP

The incidence of clinical symptoms and physical signs is presented in Fig 3 Every patient had abdominal pain and/or pain in the extremities at the beginning of the attack The symptoms were similar

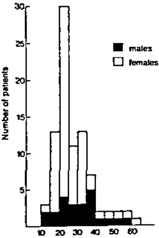


Fig 2 Age distribution of 86 patients at the first acute attack

Table III *Faecal excretion of coproporphyrin and protoporphyrin and coproporphyrin isomer distribution in acute intermittent porphyria (AIP) and variegate porphyria (VP)*

	No of cases	Coproporphyrin (µg/g dry weight) Mean	Range	Coproporphyrin III (% of total) Mean	Range	Protoporphyrin (µg/g dry weight) Mean	Range
AIP							
Acute	6	33	19–49	38	30–42	78	41–120
Latent	76	17	1 3–120	44	23–69	55	5 0–330
VP							
Acute	5	700	180–1 400	67	43–70	1 120	360–2 900
Latent	34	200	11–1 200	79	58–91	380	21–2 000
Controls	30	7 3	(S D 5 5)	46	(S D 13)	18	(S D 9 0)

patient and his relatives were not co operative which hindered further studies In spite of an intensive search lasting several years in collaboration with the Dermatological Clinic of the University of Helsinki only 9 cases of porphyria cutanea tarda and a single case of erythropoietic protoporphyria were found In the latter case both parents and all relatives examined had a normal porphyrin pattern in blood and excreta Another case with erythropoietic protoporphyria has been reported in a Finnish family by Hopsu Havu et al (19)

Genetic aspects The population structure in Finland was stable with national and regional isolation until the 20th century (36) In such circumstances rare hereditary diseases tend to accumulate in limited areas This is the case for example with cystic liver disease with retinoschisis and with a syndrome that includes familial amyloidosis corneal lattice dystrophy and cranial neuropathy (37) We found the porphyric kindreds to be clustered to 5–6 areas in Finland This may suggest that genes leading to AIP and VP were formed or imported already in the early days of Finnish history and that the mutation frequency has been low

Precipitating factors The precipitating factors preceding the symptoms did not differ from earlier reports (10 46 47) Barbiturates infection sulpha and alcohol were the most usual factors An agent not earlier reported to be associated with porphyria is fern extract formerly commonly used as an anti helminthic drug Pregnancy has been stated to be dangerous for the patient with AIP (20 49) However, according to other reports it has only a minor or no effect at all (2 43) We also found the acute attack to be rare during pregnancy

The acute attack The sex difference age distribution and symptoms of our patients did not differ significantly from earlier reports (9 13 43 51) The well known syndrome of abdominal pain dark or red urine mental symptoms and peripheral pareses (13) was also the most usual one in our patients Non abdominal pain as a symptom of acute porphyria is stressed less In most patients however, the pain in the proximal parts of the extremities and also in the back was a typical symptom and may be a good hint for the diagnosis of porphyria

Skin symptoms About 80% of the patients with VP in South Africa suffer from fragility of the skin which in many cases is a severe and distressing symptom whereas acute light sensitivity is uncommon (8 9) In the European VP patients the skin symptoms are mild or completely lacking (15 52 58) Also in our patients skin fragility was slight and was present in only half of the patients Sensitivity to sunlight was completely absent In the screening test for light sensitivity however an abnormal reaction was seen in half of the patients studied

The quantitative difference between the Finnish and the South African VP patients in the severity of skin symptoms is most probably due to environmental factors According to Waldenström and Haeger Aronsen (52) porphyric patients need strong exposure to sunlight to develop skin sensitivity Theoretically calculated maximal solar irradiance based on the different latitudes is less than 10% weaker in Finland than in South Africa in mid summer (42) On the other hand there is a great difference in the duration of solar radiation strong sunshine lasting throughout the year in South Africa but only a few months in Finland Moreover in Finland even in summertime the number

of cloudy days is much greater than in South Africa (5 33) Thus the more pronounced skin symptoms in South Africa can be due to a chronic effect of sunlight This is supported by the studies of Burnett and Pathak (4) who found that a chronic exposure to light increased the porphyrin excretion in patients with porphyria Also the worsening of the skin fragility in late summer in some of our patients supports this idea

Biochemical findings The excretion pattern of porphyrins and their precursors in our patients did not differ from earlier reports (6 9 16 44 56) There were usually no difficulties in differentiating AIP and VP by means of careful urinary and faecal analysis especially in the latent stage There was however an overlap in individual values between the two types This was usually not a hindrance for the diagnosis In AIP a moderately elevated faecal porphyrin excretion was usually associated with very high urinary PBG excretion In VP on the other hand a moderately elevated PBG excretion was noted in patients with high faecal porphyrin values

Serum hemopexin which is a porphyrin binding protein (23 25) was at a normal level both in patients with AIP and with VP Normal hemopexin concentration in AIP has been reported by Muller Eberhard et al (35)

Prognosis The mortality in acute porphyric attack in our material was about 30% which is in agreement with earlier reports (6 12, 45) The mortality rate has decreased in recent years (43) evidently due to advances in intensive care Informing the patients about precipitating agents will also considerably improve the prognosis and diminish the occurrence of symptoms Only 14% of our patients who survived an acute attack had later porphyric symptoms requiring hospital admission during a follow up of about 9 years This observation is in agreement with the recent report of Goldberg s group (1) The prognosis is even better if porphyria is diagnosed in the latent stage

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Relationship between Intracellular Cyclic AMP and Lipolysis in Human Adipose Tissue

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ABSTRACT Human subcutaneous adipose tissue has been incubated in vitro in the presence and absence of isoprenaline (ISNA) The tissue concentration of cyclic AMP (cAMP) and the release of glycerol into the incubation medium were measured after various incubation periods In the presence of ISNA (6×10^{-5} mol/l), the tissue concentration of cAMP reached a peak after around 10 min and then declined to a level significantly lower than that at the start of the incubation In contrast, the ISNA induced rate of lipolysis was a linear function of the incubation time The addition of propranolol (13 µmol/l) at different times *after* ISNA did not influence the rate of lipolysis although it resulted in a decrease in the tissue level of cAMP There was a positive correlation between the maximal increase in tissue cAMP and the rate of lipolysis in adipose tissue exposed to ISNA, both in individual experiments and in a group of 23 persons No correlation was found between the rate of lipolysis and the tissue level of cAMP in adipose tissue incubated under basal conditions The findings are compatible with the theory that the β adrenergic induced lipolysis by human adipose tissue is a function of the maximal rise in the concentration of tissue cAMP It is concluded that this peak level of cAMP represents a single compartment of the nucleotide

Although it is generally accepted that the hormone induced breakdown of triglycerides in adipose tissue is mediated via the cyclic 3 5 adenosine monophosphate (cAMP) (23) there is no evidence for a quantitative relationship between the rate of lipolysis and the change in the intracellular level of cAMP (6 10) Suggested explanations of this have been either a compartmentalization of cAMP (8 11 18 19 20 26) or a pronounced sensitivity of the lipolytic system to small or undetectable changes in the level of cAMP (10 12) Alternatively it has been proposed that the lipolytic activity is correlated with the adenyl cyclase activity rather than with the level of cAMP (21) Most of these studies have been performed on isolated fat cells of the rat

The present study was undertaken to explore if there was a quantitative relationship between the concentration of cAMP and the rate of lipolysis in human adipose tissue A specific method permitting accurate measurements of cAMP in biopsy specimens has been worked out for this purpose (4)

MATERIAL AND METHODS

Human subcutaneous adipose tissue was obtained from 23 patients of both sexes aged 25–45 years undergoing cholecystectomy or operation for hernia or benign ovarian cysts They were otherwise healthy and of normal weight

The anaesthesia was induced by Narkotal® (Astra Sweden) and maintained by Leptanal® (Leo Sweden) The patients had fasted overnight and only saline was given until adipose tissue was removed at the start of the operation In some experiments subcutaneous adipose tissue was obtained under local anaesthesia from 10 obese otherwise healthy patients of both sexes Their mean age was 29 years and mean weight 210% of the ideal according to tables computed by the Metropolitan Life Insurance Company (28) The adipose tissue was transported to the laboratory in saline and divided into sections of approximately 50 mg each

Chemicals

cAMP binding protein and [^{3}H]-adenosine 3 ,5 monophosphate cyclic ammonium salt (spec act 15 mCi/mmol) were obtained from Boehringer Mannheim s kit assay" for cAMP (Cat No 15289) Sodium acetate (NaAc) trichloracetic acid and hydrochloric acid (HCl) were of Suprapure® grade and obtained from Merck West Germany Diethyl ether (Mallinckrodt®) was purchased from Kebo Sweden Protein kinase inhibitor was prepared by the method of Appleman et al (1) as modified by Gilman (15)

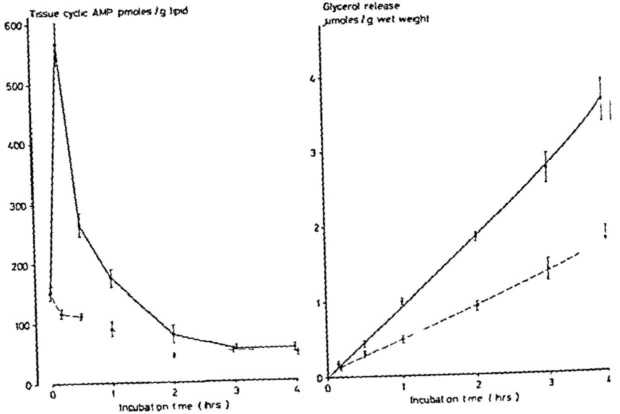


Fig 1 Effects of isopropylnoradrenaline (ISNA 6×10^{-8} mol/l) on tissue cyclic AMP levels and glycerol release ISNA was added at 0 time and pieces of human subcutaneous adipose tissue were incubated for periods as indicated in the Figure ----- =basal values (no ISNA present) Mean ±S E M $n=4$

Incubation procedures

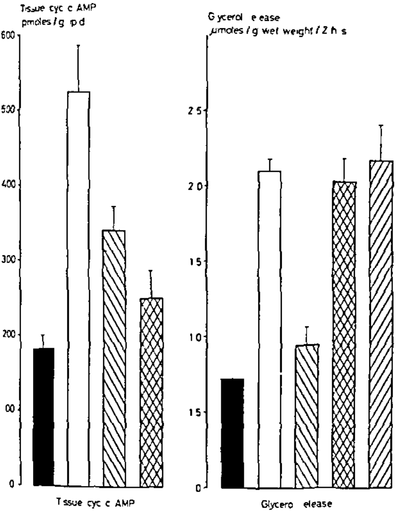
In the experiments in which the rate of lipolysis in adipose tissue was determined sections of tissue were preincubated for 30 min in Krebs Henseleit bicarbonate (KHB) buffer containing 40 mg/ml of bovine serum albumin (Armour Pharmaceutical Comp Eastbourne England Lot No R970) The pH of the buffer was adjusted to 7 4 Adipose tissue about 100 mg was then incubated in 1 ml of medium of the same type as above in polyethylene vials After incubation two aliquots (0 1 ml) of the medium were removed for glycerol determination as described by Wieland (30) and modified by Chernick (9) The significance of the use of glycerol release as an indicator of the rate of lipolysis (3) and the influence of local anaesthetic agents on lipolysis (2) have been discussed previously

When the intracellular cAMP concentration was determined adipose tissue was preincubated for 30 min in KHB buffer pH 7 4 containing 10 mmol/l of theophylline (ACO Sweden) and then incubated in a fresh medium of the same type Albumin was not present in these experiments since it has been shown to interfere with the assay for cAMP (4 22) Incubation and preincubation were carried out in glass vessels with 50 ml medium/g adipose tissue at 37°C in a water bath cycling at 40/min Air was used as gas phase

Agents added in vitro were 1 propranolol (ICI Great Britain) and isopropylnoradrenaline HCl (Winthrop England) Both agents were dissolved in water and added to the incubation medium in portions of 0 1 ml or directly dissolved in the incubation medium

Assay of intracellular cAMP

cAMP was determined by the protein binding method of Gilman (15) as described in detail elsewhere (4) Approximately 50 mg of adipose tissue were used for the assay The samples for the standard curve were prepared as the unknown samples The assay mixture contained 50 μl of the unknown or the standard sample (the latter containing 0–20 pmol of unlabelled cAMP) 2 μg of cAMP binding protein 20 μg of protein kinase inhibitor 0 8 pmol of cyclic [³H]AMP (0 02 μCi) and NaAc buffer (50 mM pH 4 0) The final volume was 200 μl Following incubation for 100 min at 0°C the samples were filtered and counted as described previously (15) Each unknown and standard sample was assayed in duplicate Theophylline was used in the cAMP experiments to boost the cAMP level since we have observed that cAMP could not frequently be measured in tissue pieces weighing less than 100 mg when cAMP was assayed in human subcutaneous adipose tissue without prior incubation



F g 2 Effects of sopropylnoradrenal ne ISNA 6×10⁻⁸ mol/l) and propranolol (13 μmol/l) on tissue cycl c AMP and glycerol release from human subcutaneous ad pose t ssue Propranolol was added to some in ubat on med um contain ng ISNA at 0 min (▧) after 10 min (▨) or 30 min (▨) of incubation cAMP was measured at ?0 m n □ only ISNA present ■ basal values (no ISNA present) Mean + S E M *n* 5

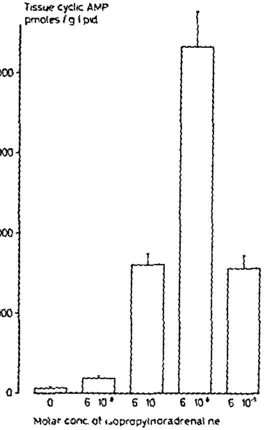
S a cal analys s

The s atistical p ocess ng of the data was made accord ng to Snedecor (?7) The means are given w th standard error of he mean (S E M) Student s unpa red *t* test and l near regress on analys s were performed

RESULTS

In the f rst set of experiments the t me relation sh p between the rate of glycerol release and the changes in the tissue level of cAMP was deter mined in subcutaneous adipose tissue incubated in the presence or in the absence of isopropylnor adrenal ne (ISNA) From ten experiments it was ascertained that the peak level of cAMP most fre quently occurred at 10-minute exposure to ISNA with a variat on of +5 m n

Fig 1 shows a typ cal experiment in wh ch the concentrat on of cAMP and the rate of l polys s were followed in ad pose tissue exposed to 6×10 ⁸ mol/l of ISNA for up to four hours of incubat on After an almost four fold increase at 10 min there was a steady fall in the concentrat on of cAMP being sign f cantly lower than the zero level at ? hours ($p<0\ 0?$) 3 hours ($p<0\ 001$) and 4 hours ($p<0\ 001$) The concentrat on of cAMP in ad pose tissue incubated in the basal med um decreased in tially and was signif cantly lower than the zero level at 1 hour ($p<0\ 0?$) as well as ? hours ($p<0\ 001$) Thereafter the level of cAMP was constant and not d fferent from that in ad pose t ssue exposed to ISNA The rate of glycerol release was a linear funct on of time in both types of incubation med um and was twice as fast from ad pose tissue exposed to ISNA S m lar t me curves for the accumula t on of cAMP have been observed in uncharted



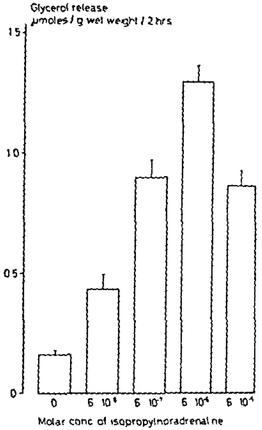


Fig 3 Effect of graded doses of isopropylnoradrenaline (0–6×10⁻⁴ mol/l) on human subcutaneous adipose tissue Cyclic AMP was determined at 10 min and the glycerol release at 2 hours Mean + S E M $n=5$

…eriments in which 10 mg/ml of albumin was used in the incubation medium The decrease in the concentration of tissue cAMP was presumably not due to loss of effect of theophylline since the theophylline induced glycerol release was linear for at least 3 hours of incubation (uncharted experiments) If theophylline was omitted from the incubation medium no or insignificant increments of cAMP were seen when adipose tissue was incubated with 6×10^{-5} mol/l of ISNA for up to 3 hours (uncharted experiments)

Fig 2 shows that the β adrenergic blocking agent propranolol produced a significant decrease in the level of tissue cAMP by 35% when added simultaneously with ISNA ($p<0.05$) and by 50% when added 10 min after ISNA ($p<0.01$) The rate of release of glycerol however was completely inhibited when propranolol (13 μmol/l) was added simultaneously with ISNA ($p<0.001$) but was not influenced when propranolol was added later than ISNA Propranolol had no effect on basal lipolysis or on the basal level of tissue cAMP (uncharted experiments)

An individual experiment (Fig 3) shows that increasing concentrations of isoprenaline up to 6×10^{-6} mol/l resulted in a gradual increase in the tissue level of cAMP as well as in the glycerol release A further increase in the concentration of ISNA to 6×10^{-5} mol/l resulted in a significantly less notable rise in the concentration of cAMP ($p<0.005$) as well as in the glycerol release ($p<0.05$) when compared with 6×10^{-6} mol/l of isoprenaline

In a group of 23 normal weight and obese subjects (Fig 4) there was a highly significant relationship between the stimulatory effect of ISNA on lipolysis and on the maximal increase of tissue cAMP ($r=+0.84$ $p<0.001$) The stimulatory effect of ISNA was calculated as ISNA induced/basal level of tissue cAMP at 10 min and ISNA/basal glycerol release at 2 hours This relationship was linear only in a semilogarithmic way (lin lipolytic effect and log effect on cAMP) When the experi-

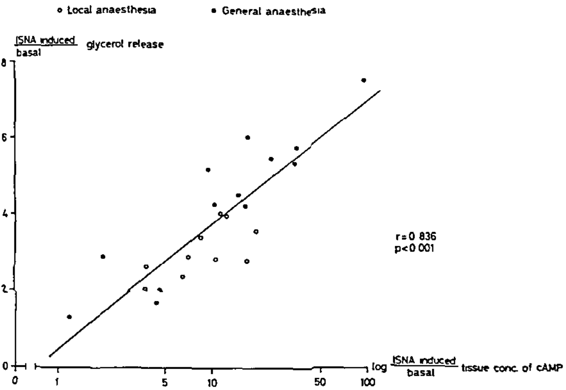


Fig 4 Relationship between the rate of stimulated lipolysis and the maximal level of tissue cyclic AMP in 13 normal weight subjects (●) and in 10 obese patients (O) Adipose tissue was incubated in the presence and absence of isoprenaline (6×10^{-6} mol/l or 6×10^{-5} mol/l) Glycerol release was determined at 2 hours and tissue cyclic AMP at 10 min of incubation from the mean of 3–6 samples

ments with the obsese and the normal weight donors were calculated separately the slopes of the regression lines were almost identical The glycerol and cAMP values were expressed as quotients (ISNA/basal) to avoid a possible influence of differences in adipose cell size A similar semilogarithmic relation between cAMP and glycerol release was observed when adipose tissue was incubated in the presence of submaximal effective concentrations of either ISNA or noradrenaline (data not shown)

No relationship existed between the basal lipolysis and the basal tissue level of cAMP in the present material when the parameters were expressed per g of lipid (Fig 5) or by cell number (uncharted experiments)

DISCUSSION

The regulation of the basal lipolysis in human adipose tissue is unknown In Fig 1 the basal lipolysis is a linear function of the incubation time whereas the concentration of tissue cAMP falls significantly during the incubation The decline in the basal level of cAMP could be due either to leakage of cAMP to the medium or to changes in the phosphodiesterase activity In Fig 5 no relationship is noted between the basal level of tissue cAMP and the basal lipolysis in subcutaneous adipose tissue obtained from a group of individuals From these data it is presumed that the basal lipolysis is regulated only partly if at all by the tissue level of cAMP

Several studies have shown that there is an early and rapid rise in the cellular level of cAMP in adipose tissue exposed to lipolytic hormones (10) Whereas there is a decline in the level of cAMP to near the initial value lipolysis proceeds at near the initial rate Fig 1 shows that in human subcutaneous adipose tissue exposed to ISNA an almost pure β adrenergic agonist tissue cAMP reached a maximal level after 10 min This is earlier than what has previously been shown in human adipocytes (5) and sections of human adipose tissue

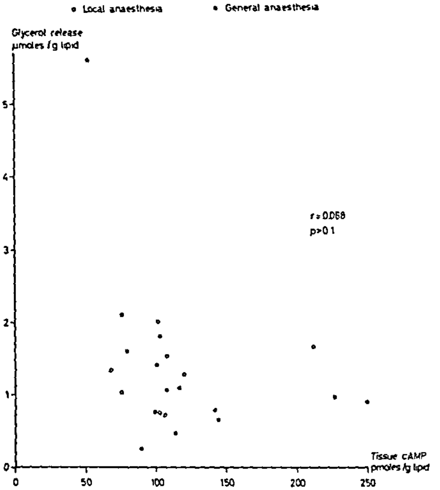


Fig 5 Relationship between basal lipolysis and level of cyclic AMP in tissue incubated with theophylline (10 mmol/l) in 13 normal weight subjects (●) and in 9 obese patients (O) Tissue cyclic AMP was determined at 10 min and glycerol release at 2 hours from the mean of 3–6 samples

13 17) and more in accordance with a recent study the [³H]-cAMP production by isolated human fat cells (16) The divergent results could be due to different experimental procedures such as the use of and the concentration of a phosphodiesterase inhibitor in the incubation medium

It has been suggested that the maintenance of stimulated lipolysis either requires a constant elevation of cellular cAMP or that once the lipase is activated it remains active in spite of decreasing level of cAMP (5) In Fig 1 cAMP after reaching the peak declined significantly below zero level and was not different from the basal level Lipolysis however was stimulated in a linear way throughout the experiment This indicates that lipolysis is maintained because of phospharylation of triglyceride lipase and not because of a constantly elevated level of cAMP

Addition of the β adrenergic blocking agent propranolol 10 or 30 min after the addition of ISNA did not alter the glycerol release although the level of cAMP was significantly decreased (Fig 2) This is contradictory to the earlier findings in isolated human (5) and rat (21) adipocytes showing that the addition of propranolol after ISNA (5) or nor adrenaline (21) reduced not only the level of cAMP but also the rate of lipolysis The data indicate that it is not likely that the maintenance of the rate of the ISNA induced lipolysis is due to a constant rise in the adenyl cyclase activity over the whole incubation period as suggested earlier (21) No changes in the rate of lipolysis were observed when the late addition of propranolol resulted in a decreased cAMP level in other words decreased adenyl cyclase activity This further supports the idea that the maintenance of lipolysis is due to the phospharylation of triglyceride lipase

Several studies on the rat adipose tissue have suggested that cAMP might be sequestered into different compartments and that only a small pool would be responsible for the transmission of the hormonal effect (8 11 18 19 20 26) The evidence

for this has been a lack of correlation between the peak level of cAMP and the rate of lipolysis (6 7 18 23) Fig 3 shows a positive although non linear relation between the ISNA induced peak level of tissue cAMP and the rate of lipolysis in an individual experiment in accordance with earlier human experiments (5 13 14) In a group of individuals this relationship was linear and highly significant as seen in Fig 4 This gives further support to the theory that the β-adrenergic induced lipolysis by human subcutaneous adipose tissue is regulated by the initial and maximal rise in tissue cAMP

It has been suggested that the range of cAMP concentration required for activating lipase is very narrow and that the hormone induced peak level of cAMP represents an unphysiological overproduction of cyclic AMP in adipose tissue (6 7 8 10 11 24 25 29) This is contradictory to the findings in the present study The close relationship between cAMP and lipolysis shown in Figs 3 and 4 indicates that in human adipose tissue the peak level of cAMP represents a single compartment responsible for the transmission of the hormonal effect

The relationship between the rate of stimulated lipolysis and the maximal level of cAMP is semilogarithmic as seen in Fig 4 This was not due to the experimental conditions with near maximal rate of lipolysis since a similar semilogarithmic relationship was observed in experiments where lipolysis was only partly activated Thus only at a low rate of lipolysis does the range of cAMP concentration seem to be narrow for activating lipase An increment from near maximal to maximal rate of lipolysis appears to require a very large production of cAMP However theophylline was used in the cAMP experiments for methodological reasons Theophylline partly inhibits the breakdown of cAMP and it is not known if this could have influenced the semilogarithmic relation between cAMP and lipolysis

ACKNOWLEDGEMENTS

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Metabolism of Mono- and Diacylglycerols in Subcutaneous Adipose Tissue of Obese and Normal-weight Subjects

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ABSTRACT Tissue monoacylglycerols (MG) diacylglycerols (DG) free fatty acids (FFA), and cyclic AMP (cAMP) and release of FFA and glycerol have been studied in vitro in subcutaneous adipose tissue of 6 obese and 7 normal weight subjects The tissue was incubated without or with 6×10^{-5} mol/l of isoprenaline (ISNA) The DG level and the fat cell volume were strongly interrelated ($r=+0.95$ $p<0.001$) The concentration of DG was increased ($p<0.05$) in obesity The changes in DG and MG were significantly interrelated ($r=+0.65$ $p<0.05$) during basal incubation ISNA increased the DG concentration in a way that was correlated ($r=+0.81$, $p<0.001$) with the ISNA induced glycerol release This indicates that 1) the basal metabolic activities of MG and DG lipase are similar and 2) DG lipase is an important rate limiting factor in lipolysis Without ISNA tissue FFA and the release of FFA and glycerol were significantly increased in the obese patients As a mean MG and DG did not accumulate in the basal state in the two patient groups The findings indicate that basal lipolysis was increased in obesity This was probably due to increased basal metabolic activity of triacylglycerol lipase since the basal cAMP levels were similar in the two patient groups In the presence of ISNA the production of FFA and the glycerol release were similar in both patient groups as was the increase in tissue DG Also the ISNA induced maximal level of cAMP was similar in the two groups With ISNA a small increment of MG was observed in adipose tissue of the normal weight subjects Taking all metabolites into account the rate of lipolysis as well as the activation of triacylglycerol lipase via cAMP in the presence of ISNA appeared to be unaltered in obesity Separate experiments with 1 ¹⁴C glycerol provided further evidence for the existence of a MG pathway for the esterification of FFA

The breakdown of triacylglycerols (TG) and the liberation of free fatty acids (FFA) to the blood stream is one of the main functions of adipose tissue Investigations of the rate of hydrolysis of TG by adipose tissue in vitro have in general been performed by determinations either of the net changes of FFA in tissue plus medium or of the net release of glycerol to the incubation medium Determination of the glycerol release is considered the most accurate index of the rate of lipolysis in human adipose tissue (7) The reason for this is that glycerol is poorly reutilized (4 18) while FFA may be reesterified to some extent The measurement of either end product of the TG breakdown has been considered justified on the assumption that the hydrolysis of TG is complete and thus occurs without accumulation of diacylglycerols (DG) or monoacylglycerols (MG) Indirect evidence for this has been given by Bjorntorp (4)

We have previously described a method to determine DG MG and FFA in the same lipid extract of human adipose tissue (?) In the preliminary experiments we observed that under certain circumstances an increase in the tissue concentration of DG took place during incubation Indirect evidence for the formation of partial acylglycerols during lipolysis in human subcutaneous adipose tissue has been presented earlier (6) and in vivo studies of FFA and glycerol turnover also indicate partial hydrolysis in human adipose tissue (7)

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Table II *Concentration of diacylglycerols (DG) monoacylglycerols (MG) and free fatty acids (FFA) in subcutaneous adipose tissue obtained from obese and normal weight patients (mean ± S E M)*

Unincubated tissue specimens were analysed immediately after removal 3–4 portions of adipose tissue were used for each assay

Type of patients	Tissue level (μmol/10⁷ cells) DG	MG	FFA
Obese ($n=6$)	20 3±3 2	10 8±2 4	20 7±1 9
Normal weight ($n=7$)	12 6±1 3	7 2±1 3	18 2±1 7
p	<0 05	NS	NS

of distilled water centrifuged and two aliquots (0 1 ml) of the supernatant were removed for the determination of glycerol (9 26) Recovery of 1 ¹⁴C glycerol added to the homogenized tissue was about 99% The addition of up to 0 5 μmol/ml of α glycerophosphate to the tissue homogenate did not influence the determination of glycerol

The utilization of glycerol by adipose tissue was measured by the method of Rodbell (20) using labelled glycerol instead of labelled glucose About 100 mg of tissue were incubated in 1 ml of medium of the same type as above with the addition of about 10⁶ cpm/ml of 1 ¹⁴C glycerol The initial and final specific activities of labelled glycerol were determined from aliquots of the incubation medium removed before and at the end of the incubation The radioactivity in CO_2 was determined as described by Rodbell (20) The radioactivity incorporated in total lipids TG DG and MG of adipose tissue was determined as described earlier (2) In these experiments labelled MG was not added to the incubated tissue for recovery purposes but to unincubated lipid extracts that were run in parallel The total conversion of glycerol into glyceride glycerol and CO_2 was calculated from the mean specific activity of 1 ¹⁴C glycerol in the incubation medium and from the amount of radioactivity found in the lipids and CO_2 The mean specific activity of labelled glycerol was calculated from the formula developed by Dole (12) knowing that the release of glycerol into the incubation medium is linear in these types of experiments (1) and assuming that the uptake of radioactive glycerol by the tissue was linear with time as has been described in rat adipose tissue (16)

The intracellular cyclic AMP (cAMP) was assayed by a modification (3) of the protein binding technique of Gilman (14) using a protein kinase inhibitor Theophylline (10 mmol/l) was added to the incubation and preincubation medium to inhibit phosphodiesterase The method and the incubation procedures have been described in detail previously (3) From several experiments it was ascertained that the maximal increment of the tissue level of cAMP occurs after about a 10-minute exposure to adrenergic stimulating drugs (1 28)

All incubations and preincubations were carried out at 37°C in a water bath cycling at 30/min Air was used as gas phase Isopropylnoradrenaline HCl (ISNA) obtained from Winthrop England was added in vitro It was directly dissolved in the incubation medium in a final concentration of 6×10⁻⁵ mol/l known from previous studies to have a maximal effect on the rate of lipolysis (13)

Radioactive assay

Samples were counted in a Tri Carb Liquid system (Packard Instrument Co) Insignificant quenching was observed

Chemicals

1 ¹⁴C glycerol (spec act 27 mCi/mmol) was obtained from The Radiochemical Centre Amersham England and purified as described by Herrera and Ayanz (16) Glycerol 1 mono-9 10-³H palmitate (spec act 45 5 Ci/mol) and 9 10-³H palmitic acid (spec act 200 Ci/mol) were purchased from New England Nuclear Corp USA and purified by thin layer chromatography (8) cAMP binding protein and ³H adenosine 3 5 monophosphate cyclic ammonium salt (spec act 15 mCi/mmol) were obtained from Boehringer Mannheim s kit assay for cAMP (Cat No 16289) Protein kinase inhibitor was obtained from Sigma USA Sodium acetate trichloracetic acid and hydrochloric acid were of Suprapure® grade and purchased from Merck West Germany Glycerol free potassium hydroxide silica gel with particle size 0 05–0 20 mm and silica gel G were also obtained from Merck Organic solvents were all reagent grade and redistilled prior to use

Determination of cell size

Two fat specimens weighing about 30 mg each were used for the determination of the cell diameter as described by Sjostrom et al (23) One hundred cells were used for the calculation of the average diameter and S D of the diameter according to the formula of Hirsch and Gallian (17) The number of fat cells incubated was calculated using the mean cellular lipid content and the total lipid content of the fat portions

Statistical analyses were performed as described by Snedecor (24) The means are given with the standard error of mean (S E M) Student s paired or unpaired t test and linear regression analyses were performed

RESULTS

The mean tissue concentration of DG in unincubated adipose tissue was significantly higher ($p<0\ 05$) in the obese than in the non obese patients (Table II) and a strong relationship ($r=+0\ 95$ $p<0\ 001$) existed between the mean cell volume and the amount of DG expressed per cell number (Fig 1) The tissue levels of FFA and MG did not differ between obese and normal weight subjects (Table II) although some tendency to positive correlation with increasing cell size appeared from the regression analyses (Fig 1) The mean content of tissue DG when expressed per g was 2 4 μmol/g lipid for

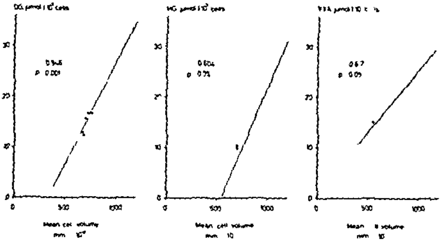


Fig 1 Relationship between the mean fat cell volume and the tissue levels of diacylglycerols (*DG*) monoacylglycerols (*MG*) and free fatty acids (*FFA*) Subcutaneous adipose tissue of 6 obese and 7 normal weight patients was analysed without prior incubations 3–4 specimens were used in each assay

the whole material and the concentrations of FFA and MG were 2 8 and 1 3 μmol/g respectively *No differences were noted between obese and* normal weight subjects when those lipids were calculated per g of adipose tissue

Table III shows that after incubation for 2 hours in basal medium the rate of lipolysis was more prominent in adipose tissue of obese than of non obese patients as judged from the final tissue FFA levels as well as from the net release of FFA and glycerol As compared with the initial levels of DG and MG (Table II) there were on an average no changes in the concentration of partial acylglycerols in adipose tissue incubated in basal medium in either group In the presence of ISNA the final concentrations of tissue FFA as well as the release of FFA to the medium tended to be more pronounced in the obese group without reaching statistical significance The true effect of ISNA (ISNA–basal) on the total production of FFA (tissue+medium) was 67 3 μmol/10⁷ cells/2 hours for the normal weight and 64 2 for the obese patients and thus not different The glycerol release in the presence of ISNA increased at similar rates in both groups The true effect of ISNA (ISNA–basal) was 16 5 μmol/10⁷ cells/2 hours of glycerol for the normal weight and 11 0 for the obese subjects This difference was not significant In the presence of ISNA the maximal increment of the tissue level of cAMP was of the same order of magnitude in both groups (Table IV)

It is also obvious from Table III that the concentrations of tissue DG were significantly higher in the adipose tissue when exposed to ISNA than when incubated in basal medium both in obese ($p<0\ 005$) and in normal weight patients ($p<0\ 001$) The increments of DG induced by ISNA were of the same order of magnitude in both groups A small *but probably significant increase* ($p<0\ 05$) in the tissue level of MG was found *in* adipose tissue of

Table III *Final tissue levels of diacylglycerols (DG) monoacylglycerols (MG) and free fatty acids (FFA) and net release of glycerol and FFA from subcutaneous adipose tissue incubated in the absence and in the presence of isoprenaline (ISNA 6×10⁻⁵ mol/l) (mean ±S E M)*

Four to six incubations were used for each type of experiment P=paired (basal vs ISNA) P_1=unpaired statistical analysis (obese vs normal weight)

	Tissue (μmol/10⁷ cells)								
	DG			MG			FFA		
	Basal	ISNA	P	Basal	ISNA	P	Basal	ISNA	P
Obese ($n=6$)	21 5±2 0	26 2±2 2	<0 005	10 0±2 2	10 0±2 9	NS	29 0±2 4	60 0±7 0	<0 0[illegible]
Normal weight ($n=6$)	13 8±1 2	19 8±0 8	<0 001	6 8±1 6	10 7±2 0	<0 05	21 6±[illegible] 0	48 7±2 2	<0 0[illegible]
P_1	<0 01	<0 025		NS	NS		<0 05	NS	

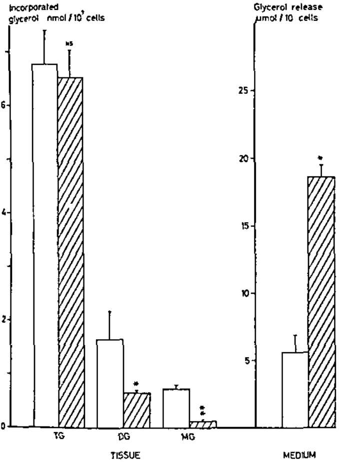


Fig 2 Incorporation of glycerol into neutral lipids Subcutaneous adipose tissue of one obese subject (no 4) was incubated for 2 hours in a 1 ¹⁴C glycerol containing medium without (□) or with (▨) the addition of 6×10⁻⁸ mol/l of isoprenaline and release of glycerol and the uptake of labelled glycerol into total lipids diacylglycerols (*DG*) and monoacylglycerols (*MG*) were determined Uptake in triacylglycerols (TG) was calculated as total lipids—(DG+MG) Statistical symbols are $*p<0.01$ $**p<0.001$ NS=not significant $n=5$ (Mean ±S E M)

the normal weight group when exposed to ISNA in contrast to the obese group

In both groups the ISNA induced net production of FFA in tissue and medium was higher than could be expected from the release of glycerol (Table III) This could only partly be explained by the accumulation of partial acylglycerols The tissue concentration of glycerol and the utilization of glycerol were studied to investigate further the discrepancy between FFA data and glycerol data

Medium (µmol/10⁷ cells/2 h)					
Glycerol			FFA		
Basal	ISNA	*P*	Basal	ISNA	*P*
8 [illegible]	19 1±3 7	<0 02	30 6±5 6	63 9±6 9	<0 02
3 1±0 5 <0 05	19 9±3 0 NS	<0 005	13 7±2 9 <0 025	54 3±10 3 NS	<0 02

No significant changes in the tissue levels of free glycerol were seen either in adipose tissue incubated under basal conditions or in the presence of ISNA Only traces of glycerol were oxidized in adipose tissue incubated under basal conditions or in the presence of ISNA In all experiments the amount of glycerol converted to neutral lipids was about 1 000 times less than that released to the incubation medium Fig 2 shows an experiment in which the conversion of glycerol into glyceride glycerol of adipose tissue of one obese subject was studied In the basal state about 80% of the utilized glycerol was recovered as TG about 15% as DG and about 5% as MG The addition of ISNA in vitro did not change the amount of glycerol that was transferred into TG However the conversion of glycerol into DG and MG was significantly reduced (by 60% and 90% respectively) when ISNA was present

In adipose tissue incubated in a basal medium for 2½ hours the concentration of DG decreased in

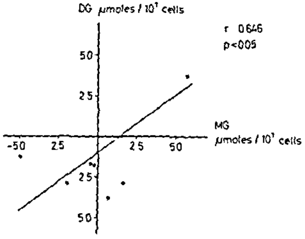


Fig 3 Relationship between changes in the tissue levels of diacylglycerols (*DG*) and monoacylglycerols (*MG*) during basal incubation Subcutaneous adipose tissue was preincubated for 30 min and then incubated in a fresh medium of the same type for 2 hours Initial and final determinations were made and the net changes of DG and MG were calculated 3–5 specimens for each assay

most patients and that of MG also decreased in several experiments (Fig 3) For the whole material the changes in DG and MG were positively and significantly interrelated ($r=+0\ 65$ $p<0\ 05$)

In the total material there was a significant and positive relationship ($r=+0\ 81$ $p<0\ 01$) between the increment in the tissue concentration of DG induced by ISNA (ISNA–basal) and the rate of ISNA stimulated glycerol release (ISNA–basal) (Fig 4) A similar but weaker relationship was observed between the effects of ISNA on tissue MG and the rate of glycerol release ($r=+0\ 59$ $p<0\ 05$) but not between the changes in tissue MG and the glycerol release On the contrary ISNA induced changes in the partial acylglycerols were not correlated with mean fat cell volume

DISCUSSION

Metabolism of partial acylglycerols

When adipose tissue was incubated in a basal medium the small changes of tissue DG and MG were parallel (Fig 3) Thus an increase or a decrease in DG was accompanied by a concomitant change in MG This indicates that the basal metabolic activities of DG and MG lipase are of similar nature in human adipose tissue There was no difference in the mean concentration of partial acylglycerols before and after basal incubation in adipose tissue of obese or normal weight subjects (Tables II and III) This suggests that DG and MG lipase are not important rate limiting factors for the basal lipolysis

On the contrary the findings with adipose tissue incubated with a maximally effective concentration of ISNA (Fig 4) indicate that the metabolic activities of DG and MG lipases do not run parallel during stimulated lipolysis In the presence of ISNA the release of glycerol above the basal was significantly and linearly correlated with the effect of ISNA on the tissue concentration of DG but not on that of MG It can be calculated from Fig 4 that when 20 μmoles of TG were completely hydrolyzed about 5 μmoles were partially hydrolyzed to DG Thus not only TG lipase but also DG lipase seems to be an important rate limiting factor

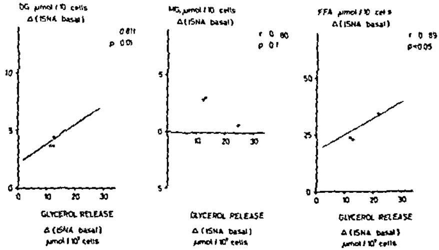


Fig 4 Relationship between the true effect of isoprenaline (=Δ (*ISNA–basal*) on glycerol release and on the tissue levels of diacylglycerols (*DG*) monoacylglycerols (*MG*) and free fatty acids (*FFA*) Subcutaneous adipose tissue of 6 obese and 6 normal weight patients was incubated for 2 hours with or without 6×10^{-8} mol/l of ISNA $n=4$–5 for the individual experiments

Table IV *T ss e le el of cycl c AMP n s b c taneo s ad pose t ss ie from obese and normal we gl t pat e ts nc b te l tl t and tl tl e presence of soprenal e (ISNA 6×10⁻⁵ nol/l) (mean +S E M)*

De e mined at 10 m n of ncuba on from the mean of 4-5 t ue p eces

	Cycl c AMP (pmol/10 cells)		
	Con ol	ISNA	ISNA control
Obe e (n 6)	7 9+1 0	7 85?+ 979	7 1 4+? 931
Normal we ght (n 6	1 056+256	9 000+2 639	7 943+? 335
p	NS	NS	NS

at least in the maximally st mulated l polysis In crements of DG and of MG during st mulated l polys s are hardly explained by accelerated synthesis of part al acylglycerols since the esterif cat on of FFA by human subcutaneous ad pose tissue in a glucose free incubat on med um is negl g ble (? 19)

The concentrat on of DG was strongly correlated wi h the fat cell volume (Fig 1) and increased in obese pat ents (Table II) On the other hand the ISNA nduced increment of tissue DG was of the same order of magn tude in ad pose t ssue of obese and normal we ght subjects (Table III) A weak relat onsh p existed also between the level of MG and the fat cell volume (F g 1) In the presence of ISNA there was a small increase in the concentra t on of MG in ad pose t ssue of the normal we ght pat ents but not of the obese (Table III) Th s m ght nd cate that the metabol c activity of MG l pase but not of DG l pase is increased n the obese state

Quan at e aspects of l poly s s

It appears from Table III that when calculated per cell number the basal rate of l polys s was in creased in the obese group as described earl er (15 29) Th s m ght be due to increased basal meta bol c act v ty of TG l pase in obes ty since the basal levels of cAMP were sim lar n ad pose t ssue of obese and normal we ght donors (Table IV) Tables II and III show that the average basal breakdown of TG was complete in both groups and thus occur red w thout accumulat on of lower acylglycerols

As judged from the t ssue levels of FFA and the rate of FFA and glycerol release in Table III both total l polys s in the presence of ISNA and ISNA nduced l polys s over the basal ere s milar in the obese and the normal we ght group Th s f nd ng is in agreement with earl er reports (5) but contrad c tory to a recent report by us show ng ncreased lipolyt c response in stimulated cond t on in obes ty (?9) The d vergent results could be due to the small cl n cal material in the present study and to the small d fference in the fat cell s ze of obese and normal e ght pat ents (Table I) Even hen the ISNA induced accumulat ons of DG and MG ere taken nto account with the ISNA nduced glycerol release over the basal the rate of st mulated l poly sis appeared to be equal in the two pat ent groups As judged by the t ssue levels of cAMP presented in Table IV also the activat on of hormone sensi tive TG l pase appeared to be of the same order of magn tude n ad pose tissue of obese and normal weight subjects

It can be calculated from Table III that the rat o of the true effect (ISNA basal) of ISNA between the glycerol release and the total product on of FFA (tissue med um) was about 1 4 n the obese and about 1 6 in the normal we ght group instead of the expected rat o of 1 3 Th s nd cates an over production of FFA in relation to the glycerol pro duct on of ad pose tissue of obese and to some extent also of normal we ght subjects s nce it could only partly be explained by partial hydrolysis of TG to DG and MG (Table III) The d screpancy be tween FFA and glycerol data was not due to ac cumulat on of glycerol in the t ssue or to the ut liza t on of glycerol Poss ble explanat ons could be that some FFA orig nated from hydrolysis of l p ds of non acylglycerol orig n such as cholesterol esters or from the breakdown of phosphat d c acid

Con ers on of glycerol to TG

Stud es of ad pose t ssue of the rat have shown that FFA are esterif ed via phosphat d c ac d to DG and TG (?5) However also a MG path ay has been postulated in ad pose t ssue of the hamster (?1 22) as well as in man (2) The experiment in F g ? shows that 1 C glycerol was converted into MG Th s could not be due to partial hydrolysis of h gh r acylglycerols s nce the incorporat on of glycerol nto MG and DG was decreased by ISNA wh le the total pool of part al acylglycerols was unchanged or increased (Table III) Instead further ev dence is prov ded of a MG pathway in human ad pose t ssue

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Urinary Cyclic AMP

Relation to Albumin corrected Serum Calcium in Healthy Persons and Patients with Primary Hyperparathyroidism

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ABSTRACT In 21 healthy volunteers, mean excretion of cyclic adenosine 3',5' monophosphate (cAMP) in urine was 4 2±1 0 (S D) μmol cAMP/24 hours 3 0±1 2 μmol/g creatinine and 3 8±1 2 μmol/24 hours/100 ml/min renal clearance of creatinine (CCr) An inverse correlation exists between the excretion of cAMP and serum calcium corrected for variations in serum albumin, most clearly demonstrated when cAMP is expressed as μmol/24 hours/100 ml/min of CCr ($r=-0\ 630$ $p<0\ 01$) In 21 patients with operatively verified hyperparathyroidism the mean urinary excretion of cAMP/24 hours was 5 0±1 9 μmol uncorrected, 4 8±1 8 μmol/g creatinine and 6 6±2 1 μmol/100 ml/min of CCr The latter two of these parameters differ significantly from the normal group ($p<0\ 001$) but conceal the fact that many patients with hyperparathyroidism excrete normal amounts of cAMP in the urine, independent of the mode of calculation However, when cAMP is correlated to albumin-corrected serum calcium this overlap between hyperparathyroidism and normality disappears completely The results support the concept that cAMP excretion is influenced to a considerable degree by the biological activity of circulating parathyroid hormone They also indicate that the simultaneous measurement of cAMP in urine and albumin-corrected calcium in serum is a useful aid in distinguishing hyperparathyroidism from the state of normality

Cyclic adenosine 3' 5' monophosphate (cAMP) is the intracellular second messenger in several hormone actions including those of parathyroid hormone (PTH) on bone and kidney (18) During this process some cAMP leaks out from cells to body fluids and urine and as much as 20–50% of urinary cAMP derives from renal production (4) Renal adenylate cyclase can be stimulated by a variety of hormones of which however PTH is the only one causing a marked increase in the urinary excretion of cAMP (5 6 11) Consequently urinary cAMP excretion is raised in some cases of primary hyperparathyroidism (PHP) and decreases in response to parathyroidectomy (11 21) Unfortunately many patients with PHP excrete normal amounts of cAMP (8 15 16 21) so in general determinations of urinary cAMP excretion have been of little diagnostic aid

The well known inverse relationship between serum calcium and concentrations of serum immunoreactive PTH in normal man (1 2) prompted this study of a similar relationship between albumin corrected serum calcium as an indirect measure of ionized calcium and the urinary excretion of cAMP which supposedly reflects levels of biologically active PTH in circulation Our results disclose an inverse correlation between these parameters in normal man and show furthermore that all patients with PHP excrete disproportionately large amounts of urinary cAMP

These results indicate that when properly used measurements of urinary cAMP are more valuable in the diagnosis of PHP than indicated in previous reports

MATERIAL

The *reference population* comprised 21 healthy volunteers 10 males and 11 females aged 17–54 years (mean 32) Thirteen were investigated on a standard diet in

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Table I *Excretion of creatinine (mean±S D) renal clearance of creatinine (CCr) and urinary cAMP i healthy volunteers hypo- and hyperparathyroid patients*

	A Hypoparathyroid (n=6)	B Normal (n=21)	C Hyperparathyroid (n=21)	Significance of difference
Creatinine (g/24 h)	0 91±0 27	1 49±0 46	1 08±0 31	A/B p<0 01 B/C p<0 01
CCr ml/min	71±15	114±29	79±25	A/B p<0 001 B/C p<0 001
cAMP (µmol/24 h)	1 8±0 7	4 2±1 0	5 0±1 9	A/B p<0 01 B/C n s
cAMP (µmol/g Cr)	2 0±0 7	3 0±1 1	4 8±1 8	A/B p<0 02 B/C p<0 001
cAMP (µmol/24 h/ 100 ml/min of CCr)	2 5±0 7	3 8±1 2	6 6±2 1	A/B p<0 001 B/C p<0 001

hospital with slightly restricted physical activity and eight on a free intake of food during normal but not excessive physical activity

The *hyperparathyroid group* comprised 21 patients 5 males and 16 females aged 21–79 years (mean 58) Due to calcium metabolic studies 16 of the patients were studied on a standard diet and only five on unrestricted food intake Subsequently 16 had parathyroid adenomas and five hyperplastic parathyroid tissue removed The urinary excretion of cAMP was never used as a criterion for operation

Six patients with *postsurgical hypoparathyroidism* one male and 5 females aged 31–58 years (mean 46) were studied on a free intake of food and their usual supplements of calcium and vitamin D Their physical activity was normal but not excessive

METHODS

e standard diet for the calcium metabolic studies was usually given for five days as four identical meals at 8 a m 1 p m 6 p m and 11 p m The calculated content of the diet was about 800 mg calcium 1000 mg phosphate and 60–140 mEq sodium/day and 1 g protein/kg b wt /day

Urine was collected during one or two 24-hour periods in plastic containers with 200 mg of aminophyllin added as a phosphodiesterase inhibitor and kept in a refrigerator at 4 C until the end of the collection period Then a specimen was removed for creatinine analysis while other samples were diluted 1/50 and 1/100 with assay buffer containing 5 mM EDTA and kept at −20 C until assayed for cAMP by a competitive protein binding technique (13) This method has an interassay variation of 7 4% Blood was drawn in the morning non fasting on each day of urine collection

Serum and urine were analyzed for calcium (Perkin Elmer atomic absorption spectrophotometer 290 acetylene air flame) and creatinine (3) Serum albumin was determined (9) and the albumin correction of serum calcium calculated based upon the regression of serum calcium upon serum albumin observed in 31 control subjects aged 20–45 years serum calcium (mmol/l)=0 86×serum albumin (mmol/l)+1 20 r=0 7? p<0 001 Serum calcium was corrected to the normal average of serum albumin of 0 720 mmol/l by the formula albumin-corrected calcium=measured calcium+((0 720−measured albumin)×1 86) (10)

The two-tailed Student s t test was used for statistical analysis

RESULTS

A group wise comparison of urinary cAMP excretion in hypo- eu and hyperparathyroidism is presented in Table I It is evident that the control group differs from the patient groups with regard to distribution according to sex age lean body mass (as reflected by urinary creatinine excretion (14)) and 24-hour clearances of creatinine (CCr) Expressing urinary cAMP per g creatinine or per standard CCr of 100 ml/min improves the discrimination between hyperparathyroidism and normality but as many as 11 and 8 patients respectively remain within normal limits despite such correction As a group *hypoparathyroid* patients excrete definitely less cAMP than normals

When the level of albumin-corrected calcium is taken into consideration urinary cAMP excretion turns out to be inversely correlated to this parameter in normal man This holds true regardless of the way in which urinary cAMP is expressed (Figs 1–3) The respective coefficients of correlation between albumin corrected serum calcium and the various measures of urinary cAMP are the follow

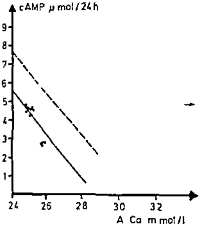


Fig 1 Urinary excretion of cAMP/24 h related to serum calcium corrected for binding to albumin ● = normal controls × patients with hyperparathyroidism = limit for highest observed normal value

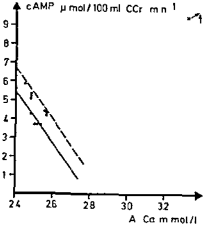


Fig 3 Urinary excretion of cAMP/100 ml clearance of creatinine/min related to serum calcium corrected for binding to albumin Symbols as in Fig 1

ing cAMP in µmol/24 h ($r=-0\ 607$ $p<0\ 01$) cAMP in µmol/g creatinine ($r=-0\ 613$ $p<0\ 01$) and cAMP in µmol/24 h/100 ml/min of CCr ($r=-0\ 630$ $p<0\ 01$) The correlation of cAMP to serum total calcium (uncorrected for binding to serum albumin) is far less the respective r values being $-0\ 351$ (n s) $-0\ 427$ (n s) and $-0\ 455$ ($p<0\ 01$) The amount of cAMP excreted probably did depend to a certain extent on diet since the respective coefficients of correlation of the standard diet group ($n=13$) were definitely higher $r=-0\ 867$ $p<0\ 001$ $r=-0\ 884$ $p<0\ 001$ $r=-0\ 817$ $p<0\ 001$

According to Figs 1–3 *all* patients with PHP excrete inappropriately large amounts of urinary cAMP when their level of albumin corrected calcium is taken into consideration

The degree of separation of normals from hyperparathyroid patients can be expressed in one figure by using a cAMP–calcium product based upon the inverse relationship between cAMP and serum calcium corrected for binding to albumin The equation used is D=(serum calcium mmol/l–2 40)× cAMP (D for discrimination) Table II summarizes the values for D in the normal and the hyperparathyroid groups D distinguishes completely between the two groups but it must be emphasized that this

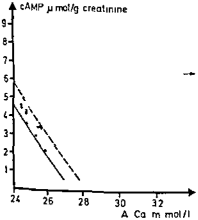


Fig 2 Urinary excretion of cAMP/g creatinine related to serum calcium corrected for binding to albumin Symbols as in Fig 1

Table II *Values for the cAMP–calcium product (D) based on the equation D=(serum calcium (mmol/l)–2 40)×urinary cAMP calculated separately for each of the three ways in which urinary cAMP may be expressed*

Median values range within parentheses Serum calcium values are corrected for binding to albumin

	D calculated on the basis of cAMP expressed in		
	µmol/24 h	µmol/g Cr	µmol/24 h/ 100 ml/min of CCr
Normal (n=21)	0 46 (0 22–0 94)	0 32 (0 11–0 56)	0 41 (0 23–0 69)
Hyperparathyroid (n=21)	2 71 (1.33–7 36)	2 74 (0 73–8 45)	3 95 (1 12–13 91)

discriminant can only be used when urinary cAMP is above the lowest observed normal value

The D value is simply a means of applying a tangible figure to the cAMP–calcium relationship Equally useful information can be obtained by plotting the raw data directly on a graph of the two criteria

DISCUSSION

Urinary cAMP consists of two moities filtered cAMP derived from circulation and cAMP produced within the kidney Therefore the major determinants of urinary cAMP encompass 1) the concentration of cAMP in plasma 2) the glomerular filtration rate 3) the total number of receptor sites for PTH in the kidney and 4) the concentration of biologically active PTH in plasma Usually urinary cAMP excretion is expressed in three ways none of which is entirely satisfactory They are the uncorrected urinary excretion of cAMP the urinary excretion of cAMP/g creatinine (as an indirect measure of lean body mass (14)) and the urinary excretion of cAMP/standard CCr (19) As far as normal man is concerned we find that regardless of the way of expressing urinary cAMP this excretion is inversely correlated to the concentration of albumin-corrected serum calcium This observation lends further support to the concept that a major fraction of cAMP in urine stems from renal production through the action of PTH on specific kidney ...rs for this hormone (7 11 17 20) As most ...s for immunoreactive PTH in serum do not ...ognize the biologically active N terminal part of ... hormone (1) measurement of urinary cAMP representing a biological receptor assay is an additional tool in the investigation of disorders of calcium metabolism

Comparing hyperparathyroid patients a group dominated by middle aged or elderly females with a group of relatively young control subjects we find like other investigators (15 16 19) that urinary cAMP excretion corrected for variations in lean body mass and CCr offers a better distinction between hyperparathyroidism and normality than does the uncorrected excretion Most evident however is the fact that all patients—regardless of the mode of expression—excrete more cAMP than one would expect from the normal interrelation of albumin-corrected serum calcium and urinary cAMP excretion (Figs 1–3) This observation agrees well with that of Arnaud et al (1) who found inappropriately high although not definitely supranormal levels of serum immunoreactive PTH in many hyperparathyroid patients

When other causes of increased urinary cAMP excretion such as hyperthyroidism (12) diabetes mellitus (22) and metabolic alkalosis (7) have been ruled out the combined determinations of urinary cAMP excretion and serum albumin-corrected calcium can be used as an aid to differentiate between PHP and normality

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Pure Mitral Regurgitation

Etiology Pathology and Clinical Patterns

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ABSTRACT The varied etiology of pure mitral regurgitation is demonstrated in this clinicopathological study, comprising 59 surgically treated cases with this condition One third of the cases was of rheumatic origin, one fifth had ischemic heart disease, another fifth floppy valves and one eighth an isolated rupture of the chordae with necrosis of the chord matrix To our knowledge the histopathological findings in the last group have not been described before Congenital mitral regurgitation bacterial endocarditis and cardiomyopathy were rare causes of mitral regurgitation Differences between the groups were observed in the sex ratio duration of history, auscultatory findings, ECG signs compliance of the left ventricle and in the morphological findings

During the last ten years the availability of the mitral valve prosthesis has made surgery for mitral regurgitation a commonly used and effective method of treatment

The purpose of the present paper is to review 59 cases of surgically treated pure mitral regurgitation Based on histopathology the cases are classified in etiological groups and this classification is correlated to the clinical patterns presented

MATERIAL AND METHODS

Clinical material

In the period 1965–73 241 patients underwent open heart surgery for dysfunction of the mitral valve at Surgical Department A University Hospital Oslo Norway As judged from pre and peroperative findings 72 patients had pure mitral regurgitation without accompanying stenosis Severe mitral regurgitation and class III or IV functional capacity (N Y H A) were the indications for operation All patients had been investigated with PCG 12 lead ECG right and left heart catheterization and left ventricular cineangiograms

Mitral valve prostheses were inserted in 64 patients while 8 underwent repair surgery only Valve tissue for histological examination was available in 59 of the 64 cases This report deals with the clinicopathological correlations in these 59 patients classified clinically before the study as shown in Table I All ECGs were reexamined Q waves of more than 0 04 sec in duration and 0 4 mV in depth were defined as pathological

The X ray findings were based on reports from an experienced cardiac roentgenologist The heart size was measured as described by Amundsen (2)

The clinical diagnosis of rheumatic heart disease was based on known arthritis or chorea minor together with carditis The diagnosis of ischemic heart disease was based on known myocardial infarction diagnosed in hospital and/or typical angina on effort and/or distinct pathological Q waves in the ECG One of the patients with congenital mitral insufficiency had atrial septum defect of the primum type The other was diagnosed at operation and was found to have cleft mitral valve The diagnosis of bacterial endocarditis was made from blood cultures and the clinical picture Idiopathic hypertrophic subaortic stenosis was diagnosed by left heart catheterization with angiocardiography

Histopathological methods

The investigation was undertaken on a retrospective basis Accordingly the macroscopical descriptions of excised valves and the material available for microscopical evaluation were not standardized All sections were stained with hematoxylin phloxine saffron and the slides were reviewed twice by the pathologist without knowledge of the clinical peroperative or macroscopical findings The following morphological tissue changes were recorded fibroelastic hyperplasia fibrosis of pars spongiosa nodular sclerosis calcification vascularization atheromatous change myxomatous change (focal or diffuse) necrosis presence of inflammatory cells and presence of necrotic papillary muscle (27 32 33 34)

Table I *Clinical classification of mitral regurgitation*
CMR=congenital mitral regurgitation RHD=rheumatic heart disease IHD=ischemic heart disease BE=bacterial endocarditis IHS=idiopathic hypertrophic subaortic stenosis

	n	With associated aortic valve dysfunction	Rupture of chordae tendineae	Rupture of papillary muscle	Dysfunction of papillary muscle	Floppy valves	Stretched chordae
CMR	2						
RHD	17	6					
IHD	12		4	1	4		
BE	3		1				
IHS	1						
Unknown	24	2	12	2		3	1
Total	59	8	17	3	4	3	1

Table II *Distribution of morphological findings in the clinical groups*
Abbreviations as in Table I

	Calcification	Vascularization	Diffuse myxomatous change	Necrosis	Fibrosis
CMR		1			1
RHD	2	14			1
IHD		2			10
BE		2			1
IHS					1
Unknown		4	11	7	2

Table III *Final classification of the etiology of mitral regurgitation*
FV=floppy valves IRCT=isolated rupture of chordae tendineae other abbreviations as in Table I

			Additional morphological features		
	n	%	Infarction of papillary muscle	Secondary endocarditis	Focal myxomatous change
CMR	2	3		1	1
RHD	21	36		4	16
IHD	12	20	4	2	5
BE	3	5			
IHS	1	2			
FV	13	22			2
IRCT	7	12			
Total	59	100	4	7	24

RESULTS

Based on morphological findings five patient groups were judged to be most relevant for an etiological assessment The distribution is shown in Table II Four cases in the group with an unknown etiology showed vascularization of valve tissue and the clinical picture was similar to that of the patients known to have had rheumatic fever Diffuse myxomatous change was found in 11 specimens The two specimens showing fibrosis of the pars spongiosa with only focal myxomatous change had the characteristic appearance of floppy valves at operation The seven specimens showing necrosis and scant lymphocytic infiltration all had rupture of

Table IV *Clinical pattern in mitral regurgitation of different etiologies*
Abbreviations as in Tables I and III

	N	Age (y)[a]	Men/ women	Duration of dyspnoea or severe angina (y)	Hypertension[b] (%)	Systemic embolization (%)	Dyspnea (%)	Palpitations (%)
CMR	1	55	0/1	10		100	100	100
RHD	15	52 9 (32–64)	7/8	9 4 (3 0–25)	7	7	100	93
IHD	12	56 3 (45–64)	8/4	1 1 (0 2–2 0)	25		83	25
BE	3	47 0 (20–64)	2/1	5 9 (0 1–11)		33	100	33
IHS	1	50	1/0	20			100	100
FV	11	58 6 (36–64)	7/4	6 0 (1 0–25)	18	9	100	45
IRCT	7	57 3 (52–69)	5/2	7 0 (0 3–10)	14		100	57

[a] Range within parentheses [b] BP>165/95 mmHg

Table V *Auscultatory findings in pure mitral regurgitation*
Abbreviations as in Tables I and III

	Murmur over all grade	Midsystolic accentuation (%)	Radiation to 2nd left or right intercostal space (%)	Ventricular gallop rhythm (%)	Accentuated pulmonary component (%)
CMR	5			100	100
RHD	3–4			87	73
IHD	2–4	33	33	75	83
BE	3–5	33	33	100	100
IHS	3	100	100	100	100
FV	3–5	55	55	82	82
IRCT	3–4	57	57	86	86

Table VI *Laboratory data in pure mitral regurgitation*
LVEDP=left ventricular end-diastolic pressure other abbreviations as in Tables I and III Figures within parentheses= range

	ECG findings		X ray findings		Hemodynamic findings	
	Sinus rhythm (%)	Path Q waves (%)	Heart size (ml/m² BSA)	Atrial size	Cardiac index[b]	LVEDP (mmHg)
CMR			980	2 0	2 0	14
RHD			863 (625–1 270)	2 7 (2–3)	2 5 (1 8–3 4)	10 (4–15)
IHD	75	67	679 (425–960)	1 8 (1–3)	2 3 (1 8–3 1)	20 (8–32)
BE			873 (770–930)	3 0 (3)	2 4 (1 9–2 8)	14 (10–20)
IHS			740	2 0		10
FV	45		732 (515–930)	2 2 (1–3)	2 2 (1 8–3 0)	17 (0–23)
IRCT			816 (610–1 000)	2 4 (1–3)	2 4 (1 7–3 2)	14 (4–27)

1=normal 2=large 3=very large
[b] Determined by the Fick method

the chordae tendineae Table III shows the final etiological classification and also the distribution of complicating endocarditis and focal myxomatous change

The clinical auscultatory ECG X ray and hemodynamic data are summarized in Tables IV–VI Patients with aortic dysfunction or atrial septal defect have been excluded

DISCUSSION

Congenital mitral regurgitation

Congenital mitral regurgitation is often associated with defects such as coarctation of the aorta (5) patent ductus arteriosus (43) transposition (25) atrial primum septum defect or endocardial fibroelastosis (17 42) Atrial primum septum defect caused mitral regurgitation in one of our patients Isolated congenital mitral regurgitation was found in one patient who had a cleft mitral valve He was 55 years old when mitral valve replacement was performed with symptoms and signs similar to patients having rheumatic mitral regurgitation The valve tissue showed fibrosis while the specimen from the ASD primum case revealed signs of complicating endocarditis

Rheumatic heart disease

This group comprised 36% of our total material The etiological classification was based on clinical data supported by the finding of an unspecific inflammation with blood vessel proliferation or calcification Vessels are normally present in human valves only in fetal life (16 45) Calcification occurs frequently in damaged tissue (3) and was considered to be consistent with earlier episodes of endocarditis No Aschoff bodies were found in the valve specimens in agreement with the findings by Kern and Tucker (23) In spite of a positive history of rheumatic fever one specimen only showed fibrotic change probably due to a sampling error

dditional findings in valves with sequelae of eumatic endocarditis were a variable degree of ibrosis hyalinization and a proliferation of elastic fibres and smooth muscle cells In four specimens acute inflammation with granulocyte infiltration fibrinous exudate and necrosis were also present This was regarded as a sign of complicating subacute bacterial endocarditis although no bacteria were seen (4)

Fifteen of the 21 patients did not have additional aortic valve dysfunction and formed a rather homogeneous clinical group The age distribution was the same as for patients with combined mitral stenosis and regurgitation of rheumatic origin at this hospital but the sex distribution was different women/men 8/7 and 109/60 respectively The characteristic findings at physical examination in patients with rheumatic mitral regurgitation are well known (1 6 12 18) All patients in this category revealed a pansystolic murmur with classical location The first sound was reported as normal in 11 and accentuated in 4 All patients had atrial fibrillation which is a higher frequency than in the series of Selzer and Katayama (36) X ray evidence of cardiomegaly and particularly a large left atrium was the rule

Ischemic heart disease

Twelve patients had mitral regurgitation caused by ischemic heart disease Four of these had ruptured chordae tendineae one had rupture of papillary muscle and four had papillary muscle dysfunction In the other three patients the only pathological finding was small and atrophic chordae tendineae on cross section Eighty three per cent of the valve specimens revealed fibrosis of the pars spongiosa (Table III) Infarcted papillary muscle was present in four of our specimens

All patients in this group developed dyspnea on effort or severe angina less than 2 years before mitral valve insertion Two of the patients had only a grade 2 murmur Three of the four patients with rupture of the chordae tendineae had a pansystolic murmur with an epicenter near the apex with radiation towards the axilla

The low number of patients in this group is in contrast to the high frequency of mitral regurgitation murmur in myocardial infarction reported by Heikkila (22) and also to the frequency of clinically severe mitral regurgitation observed by Braunwald (8) In a study by Sanders et al (35) only a few patients underwent open heart surgery because of the high mortality rate in the acute phase

Experimental studies in dogs have shown that isolated papillary muscle destruction does not produce mitral regurgitation while this does develop when papillary muscle destruction and myocardial infarction co-exist (29 30 43 44) Abnormal contraction of the ventricular wall probably augments malfunction of the papillary muscle (28)

In an electronmicroscopical study of ruptured and non ruptured chordae by Fenoglio and Pham (19) cardiac muscle cells could be traced in the mid portion of the chordae In a patient who had infarction of the papillary muscles these cardiomyocytes showed signs of ischemic damage It was concluded that this might be responsible for the secondary enzymatic changes including digestion of the collagen core of the chordae thus resulting in its rupture



Fig 1 Diffuse myxomatous change in valve matrix with fragmentation and disorganization of the connective tissue fibres (×335)

Bacterial endocarditis

Six patients in the total material aged 20–64 years had suffered from bacterial endocarditis Three of them had no history of previous heart disease The symptoms were similar to those of patients having ruptured chordae although only one patient exhibited rupture at operation Two of the specimens showed unspecific inflammation No bacteria were seen probably due to antibiotic therapy The third specimen only showed fibrosis but if more valve material had been embedded inflammatory changes would probably have been found

Three patients with known heart disease had suffered from complicating endocarditis whereas there were morphological signs of this complication in seven of the valve specimens

Idiopathic hypertrophic subaortic stenosis

In a study of hypertrophic obstructive cardiomyopathy Swan et al (41) reported severe mitral regurgitation in only three out of 60 patients whereas mild or moderate mitral regurgitation was found to be very common

Subsequent experience suggests that mitral regurgitation is always present when there is an outflow gradient (21) One such patient who had severe mitral regurgitation and subaortic stenosis is included in this series Valve tissue showed fibrotic change

Unknown etiology

In this group we were able to separate two distinctly different groups based on morphological findings supported by a somewhat different clinical history Patients having floppy valves with myxomatous change of valve tissue were included in one of the groups Seven specimens revealed focal necrosis of the valve matrix All of these patients had isolated rupture of the chordae tendineae and thus constituted a special group in the pathogenesis of mitral regurgitation

Floppy valves–myxomatous change

Myxomatous changes of valve–chord material with resulting mitral regurgitation and often complicated by ruptured chordae have been discussed in several papers (13 15 20 26 38) Floppy valves and diffuse mucoid valve degeneration are designations covering the same condition Such morphological specimens constituted 46% of the valves in the group having unknown etiology and 22% of the total material Cooley et al (14) reported myxomatous change of the mitral valve in 23% of their patients with pure mitral regurgitation

Myxomatous change in heart valves as a specific entity is controversial from a morphological point of view The designation includes fragmentation and disorganization of collagen and elastic fibres and an increase of amorphous ground substance giving a positive staining reaction with Alcian blue and green mucicarmine PAS and toluidine blue When most of the valve tissue is myxomatously transformed mitral regurgitation may occur clinically and the characteristic features of floppy valves are found at operation The entity was first described in 1965 and it has been speculated that this condition is Marfan s syndrome forme fruste (13 14) A specimen with these typical changes is shown in Fig 1 Two specimens that had previously been described as floppy valves at operation only revealed a focal myxomatous change The finding by Kern and Tucker (23) of a focally distributed myxomatous change in as many as 73% of the excised valves casts considerable doubt upon the statement that this is a specific pathological change In our material 41% of the valve specimens showed a focal myxomatous change distributed over almost all the groups Thus 63% of the valves were slightly or extensively myxomatous

Mitral regurgitation in this condition is probably caused by an extreme stretching of the mitral mooring elements and sometimes a straight rupture with prolapse of one or both leaflets into the left atrium Five patients had ruptured chordae two



Fig 2 Focal necrosis of valve matrix with a few mononuclear cells and nuclear debris (arrow) (×335)

showed rupture of the head of a papillary muscle three had floppy valves and one had particularly long and stretched chordae One patient had myxomatous transformation of the aortic valve as well Another one had an atrial secundum defect and floppy valves an association which has been reported earlier (7 46) Such patients may be misdiagnosed as having an atrial primum septum defect None showed signs of Marfan s syndrome (31) Nine of the patients had a short clinical history of less than three years All had a pansystolic murmur and a normal first heart sound Goodman et al (20) observed progression of a late systolic to a pansystolic murmur in one patient but this was not observed in any of our patients

Isolated rupture of chordae tendineae

This group represented 14% of the total material Histologically a peculiar type of necrosis of the valve matrix was found with small foci consisting of scattered lymphocytes with nuclear dust and fragmentation of intercellular substance as shown in Fig. 2 These changes bear a close resemblance to the lesions described by Kern and Tucker (23) as "inactive Aschoff bodies with fibrosis lymphocytes and few Anitschkow myocytes The absence of vessels the lack of a history of rheumatic fever and clinical findings different from such patients justified the basis for the group called isolated rupture of chordae tendineae The possibility that other inflammatory conditions might be involved should be considered (9 11) Virus infections of the endocardium are probably difficult to diagnose clinically Vessel proliferation in valve tissue may be weak and not necessarily present in the sectioned material Coxsackie virus infection of valves has recently been reported (10 39) Unknown nutritive or toxic factors or mechanical stress might also be responsible for the changes

To our knowledge the histopathological findings in these patients have not been described earlier In three patients studied by Selzer and Katayama (3?) "no specific inflammatory or degenerative disease" was found in resected chordae tendineae having isolated rupture

Mostly elderly men in the patient group had a comparatively long duration of symptoms Only one patient had a shorter clinical history than five years and there was no history of chest trauma The first sound was normal in six and of diminished intensity in one The ECG showed atrial fibrillation in five flutter in one and a second degree AV block Mobitz type II in one

COMMENTS

This study demonstrates the varied etiology of pure mitral regurgitation One third was of rheumatic origin one fifth had ischemic heart disease another fifth floppy valves and one eighth isolated ruptured chordae Congenital mitral regurgitation bacterial endocarditis and cardiomyopathy were rare causes There was no significant age difference between the groups It should be stressed however that these were patients with advanced mitral regurgitation and that they were operated upon because of their congestive heart failure In a series comprising medically treated patients the youngest were found in the congenital and the floppy valves groups (3?) The majority of the patients in every group was

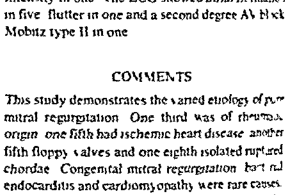


Fig 3 Left ventricular end-diastolic pressure (LVEDP) at rest in relation to cardiac size The difference between rheumatic and ischemic mitral regurgitation is obvious

male except for the rheumatic group with a sex ratio of 1/1 This is in contrast to mitral stenosis of rheumatic origin with a female/male ratio of 4/1

The shortest history of congestive heart failure was observed in the ischemic heart disease group In other studies patients with idiopathic rupture of the chordae tendineae had the shortest history of clinical symptoms (37 38 40) Our specific group of patients having isolated ruptured chordae developed heart failure over a long period indicating a progressive rupture

Midsystolic accentuation of the murmur and radiation of the murmur to the base of the heart indicated rupture or elongation of the chordae of the posterior mitral cusp As expected this was found in patients having floppy valves and isolated ruptured chordae but also in the ischemic and in the bacterial endocarditis patient groups

Contrary to other studies (24 40) none of our patients with ruptured chordae were in sinus rhythm

There was no significant difference in the degree of heart enlargement and no difference in the hemodynamic findings except for the considerably higher compliance of the left ventricle in the ischemic group compared with the rheumatic group as indicated in Fig 3

As can be seen from Fig 3 the left ventricular volume was not measured in our study To evaluate left ventricular compliance we used relative heart volume the greater part of which is made up of a dilated left ventricle in patients with mitral regurgitation It is also due to larger left atrium as shown in Table VI The larger heart volume and lower compliance in rheumatic mitral regurgitation may be due to a longer history in these patients (Table IV)

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Disopyramide in Ventricular Tachycardia

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ABSTRACT Administration of disopyramide phosphate (DE) i v in two doses 30 min apart, to a patient with ventricular tachycardia was accompanied by no, or only slight changes in systemic arterial pressure (SAP) cardiac output (Q), stroke work (SW) and pulmonary artery diastolic pressure (PADP) Heart rate fell from 123 to 103/min Following reversion to sinus rhythm which occurred 60 min after the second dose of DE at a serum concentration >4 3 μg/ml, Q and SW showed significant increases above their control values PADP fell from 20 to 6 mmHg whereas the mean SAP remained largely unchanged There seemed to be no adverse effects of drug administration In this patient recurrent attacks of ventricular tachycardia not responding to conventional antiarrhythmic treatment could be prevented by oral DE in a dose of 800 mg/day

Disopyramide (DE) administered i v has proved to have significant antiarrhythmic properties in patients with frequent ventricular ectopic beats (1 4) We have previously demonstrated (1) that the drug exerts negative inotropic effects but also that in the presence of heart failure and coronary artery disease ventricular ectopic beats can be abolished without serious side effects On the other hand there were indications that the negative inotropic effect may be of clinical importance in patients with high filling pressure of the left ventricle

A haemodynamic study was undertaken in one patient to evaluate the circulatory effects of i v DE during ventricular tachycardia in the presence of an elevated pulmonary artery diastolic pressure The case report suggests that i v DE may be valuable in converting ventricular tachycardia to sinus rhythm and furthermore that oral DE is effective in preventing ventricular tachycardia

METHODS

The methods employed in the haemodynamic study have been described in detail in a previous paper (1) DE (Rhythmodan® Roussel Lab Ltd England) was given initially in a dose of 1 7 mg/kg b wt over 2 min Thirty min later due to persistence of ventricular tachycardia a second dose of 0 7 mg/kg was administered also over 2 min The BPs in the ascending aorta right atrium and pulmonary artery were recorded continuously and simultaneously via catheters introduced from the right brachial artery and right subclavian vein respectively Cardiac output ($\dot{Q}$) was determined in duplicate by means of the dye dilution technique 5 min before and 10 30 45 120 and 180 min after the first injection of DE BP measurements and computations of the maximal derivative of the aortic pressure curve $(dp/dt)_{max}$ and of the pre-ejection period were made 5 min before at the start and 5 10 20 30 45 60 120 and 180 min after the injection The haemodynamic results are based on computer-derived data as averages of 10–20 beats A modified spectrofluorimetric method was used for DE assay (3)

CASE REPORT

A 46-year-old man presented with an uncomplicated anteroseptal myocardial infarction without serious complications and specifically no tendency to arrhythmia either in the CCU or during the first ten months following the infarction A moderate hypertension (155/110 mmHg) was however diagnosed and antihypertensive therapy with propranolol was started before the patient was discharged Eight months later mefruside was added to the antihypertensive treatment at a level of 140/105 mmHg

Ten months after the infarction the patient was admitted to hospital with a four-day history of epigastric discomfort nausea and palpitations An ECG revealed a supraventricular tachycardia with aberrant intraventricular conduction and a chest X ray showed an increased heart size (670 cm^3/m^2 BSA) with dilated pulmonary vessels Normal sinus rhythm was established by electroconversion and digoxin was substituted for the previous treatment with propranolol Because of persisting ST segment elevations in the precordial chest leads left

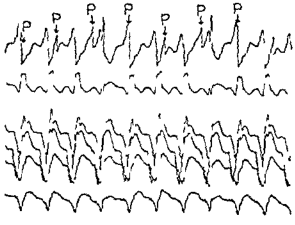


Fig 3 FCG with oesophageal lead (*top*) during ventricular tachycardia P waves are indicated

increase with each of the two DE injections but the increments were transient and followed by reductions so that the values obtained two and three hours after the first injection when the patient was in sinus rhythm were lower than the average values obtained during the preceding observation period

ECG changes

During the haemodynamic study with ventricular tachycardia there were transient moderate prolongations of the QRS intervals which were of the same order of magnitude and duration as in a previous study on the effect of i v DE in patients in sinus rhythm and with ventricular ectopic beats (1) When sinus rhythm had been established 60 min after the second injection of DE the PQ QRS and QT intervals were all within normal limits and not significantly different from the corresponding intervals of the patient s previous ECGs before ventricular tachycardia had occurred and DE had been given (Fig 2*a*)

After the commencement of long term prophylactic treatment with DE resting FCG showed a moderate prolongation of the PQ QRS and QT intervals QRS time increasing from 0 07 to 0 09 sec (Fig 2*b*) However in two exercise tests on a bicycle ergometer an intraventricular conduction defect occurred at a heart rate of about 130/min with prolongation of QRS intervals from 0 09 to 0 13 sec (Fig 2*c*) There were no subjective or objective changes associated with the appearance of the conduction defect In the first of the two exercise tests the conduction defect persisted for more than 10 min after the test had been interrupted at a heart rate of 140/min In the second test which was interrupted immediately upon the appearance of the conduction defect at a heart rate of 130/min the ECG abnormality disappeared almost simultaneously with the cessation of exercise The same type of conduction defect as that appearing in the exercise tests has been noted on two occasions in routine resting FCGs at a heart rate of 90–100/min

It cannot be excluded that DE may have contributed to the intermittent intraventricular conduction defect described above However due to the absence of signs of unfavourable side effects and because of the subjective and objective signs of antiarrhythmic effect of DE its administration has not been discontinued in spite of the latent conduction defect

DISCUSSION

Although no His bundle electrogram was recorded we consider beyond doubt on the basis of oesophageal leads (Fig 3) that the tachycardia was of ventricular origin Following administration of DE the only subjective sensation reported by the patient was dryness of the mouth This side-effect has been reported earlier (1) and can be attributed to the anticholinergic effect of the drug (4) Dryness of the mouth and chest discomfort were the only side effects specifically asked for

The haemodynamic effects following each of the two DE injections were rather small Following the first injection there was a fall in heart rate which was counterbalanced by an increase in stroke volume so that $\dot{Q}$ was largely unaffected The elevated pulmonary artery diastolic pressure showed no significant change remaining at about 20 mmHg No significant change in cardiac contractility could be deduced from the recording of $(dp/dt)_{max}$ of the central aortic pressure curve or from the calculated stroke work Transient increases in the pre ejection period suggested however that a negative inotropic effect may have occurred In a previous study on the effects of DE in patients in sinus rhythm and with premature ventricular beats (1) there were clear signs in several haemodynamic variables that DE induced a reduction of cardiac contractility It can be assumed then that any negative inotropic effect of DE in the present case was at least not aggravated by the presence of ventricular tachycardia This is even more remarkable considering that the previous study indicated an elevated

pulmonary artery diastolic pressure to be associated with a more significant negative inotropic effect than a normal pressure

The reversion to sinus rhythm did not occur until 60 min after the second DE injection The question arises therefore whether this normalization of cardiac rhythm would have occurred spontaneously without the two preceding injections Analyses of the serum concentration of DE revealed however a value of 4 3 μg/ml 30 min after the recurrence of sinus rhythm (90 min after the second injection) Previous observations (2) have demonstrated the abolition of frequent ventricular ectopic beats with this serum concentration of DE and it might be assumed therefore that abatement of ventricular tachycardia in the present case would not have occurred in the absence of DE Supporting this assumption is the fact that of the patient s eight previous episodes of ventricular tachycardia verified by ECG recordings spontaneous reversion to sinus rhythm occurred only once 24 hours after the last oral maintenance dose of DE The possibility that episodes of ventricular tachycardia occurred on other occasions with spontaneous normalization of the rhythm is unlikely due to the failure of the patient to experience the objective sensations which regularly accompanied ECG-diagnosed ventricular tachycardia in other situations

The effectiveness of DE as a prophylactic antiarrhythmic drug is strongly indicated by the fact that following eight episodes of subjectively troublesome ventricular tachycardia (with six episodes during the six months preceding DE treatment) no attack has been evident during an 18 month follow up with a regular dose not below 200 mg every 6 hours The patient claims that if on occasions the dose intervals exceed 6 hours there is a tendency for palpitations suggesting extrasystoles

The subjective sensations which have accompanied the oral prophylactic treatment with DE have so far been tolerable consisting mainly of dryness of the mouth and slight constipation The apparent effectiveness of the drug and few side effects encourage further testing of the drug in cases with ventricular tachycardia

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Clinical and Hemodynamic Findings Following Prosthetic Valve Replacement for Mitral Valve Disease

A Study of Patients with the New Bjork–Shiley Tilting Disc Valve

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ABSTRACT Clinical and hemodynamic results have been evaluated 12–24 months after mitral valve replacement with the new Bjork–Shiley tilting disc valve prosthesis After operation, most patients were improved symptomatically and were classified as I–II (N Y H A) No patient became worse Hemodynamic status at rest showed significant reduction in pulmonary capillary venous pressure, pulmonary arterial pressure and significant increase in cardiac output when compared with the preoperative values but postoperative hemodynamic abnormalities remained Exercise produced a rise in pressures in the pulmonary circuit and in cardiac output The increase in cardiac output was less than expected from the increase in oxygen consumption with a few exceptions Apparently there was no close relationship between the symptomatic improvement and the hemodynamic results Thus, the present study points to the importance of hemodynamic data in the objective assessment of the results of cardiac surgery

Prosthetic valve replacement for mitral valve disease is widely used to-day The present study was undertaken to assess the clinical and hemodynamic results after mitral valve replacement with the Bjork–Shiley disc valve prosthesis and to evaluate the relationship between clinical status and the hemodynamic findings

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MATERIAL AND METHODS

The study group comprises 33 consecutive patients with the new Bjork–Shiley tilting disc valve prosthesis with pyrolytic carbon disc occluder (3) There were 23 women and 10 men varying in age between 43 and 64 years (mean 54 5) The primary valve lesion was combined mitral stenosis and incompetence in 30 patients and pure mitral incompetence due to myocardial infarction in 3

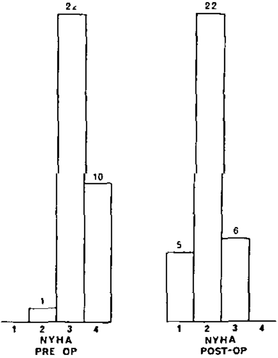


Fig 1 N Y H A functional classification before and after mitral valve replacement

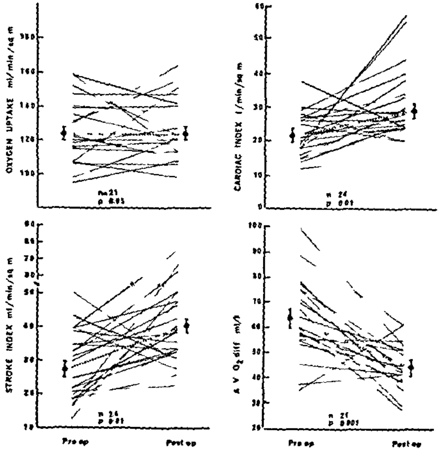


Fig 2 Hemodynamic data at rest before and after mitral valve replacement ● ♦= pre and postoperative mean values vertical bars= S E M

Operative procedures

Following sternotomy the left atrial groove was exposed while connecting the patients to a Rygg Kyvsgaard s bubble oxygenator Two-thirds of the patients were operated on during general hypothermia (30°C) crossclamping usually for two periods of 20 min interrupted by 5 min of coronary perfusion during release of the aortic clamp In one third the profound local cooling technique was applied (6) The temperature of the left ventricular wall then fell to approximately 15 C The mitral leaflets with chordae tendinae and papillary muscles were removed The largest prosthesis possible was inserted using continuous suture with a supraannular position of the prosthesis In almost one third of the patients the prosthesis was positioned subannularly The largest opening of the prosthesis was always oriented posteriorly The disc was checked to ensure free movement After closure of the atriotomy the aortic clamp was released and the patient was rewarmed Air was carefully removed from the heart After 10–15 min the heart was defibrillated The aortic crossclamping time was 40–60 min

The reexamination was performed 12–24 months after the operation At the follow up all patients were on maintenance treatment with digitoxin diuretics and anticoagulant agents Atrial fibrillation was present in 21 patients (64%) while 12 were in sinus rhythm

The pre and postoperative functional classification was carried out according to the criteria defined by the New York Heart Association Radiological heart size was determined in all cases In 28 out of 33 patients pertinent pre and postoperative hemodynamic data were available obtained by right and left sided cardiac catheterization in supine position carried out in the postabsorptive state Cardiac output was determined according to the Fick principle Blood oxygen content was determined spectrophotometrically and expired air was analyzed in a Scholander apparatus Intravascular pressures were referred to the fourth intercostal space in the anterior chest line Submaximal exercise test was carried out for 5 min with a cycle ergometer fixed to the end of the fluoroscopic table Cardiac output was determined and pressures were recorded during the final 2 min of exercise The mean diastolic pressure gradients across the mitral valve were determined by planimetric integration of simultaneously recorded pulmonary wedge pressure and left ventricular pressure after phasic correction

Left ventricular cineangiography was performed in order to evaluate the presence or absence of prosthesis insufficiency A 30–45-degree right anterior oblique view was utilized depending on the rotation which placed the prosthetic valve in profile No or insignificant regurgitation of the contrast medium into the left atrium was seen in 22 and marked regurgitation in 5 patients One patient had a significant leakage around the rim of the valve

The data are presented as arithmetic means ±S D or

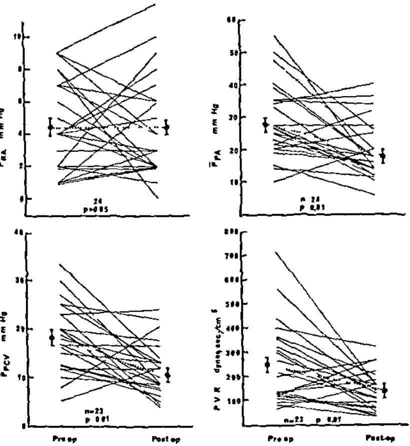


Fig 3 Pressure recordings at rest before and after mitral valve replacement
P_{RA} = right atrial mean pressure P_{PA} = pulmonary artery mean pressure
PCV = pulmonary capillary venous pressure
PVR = pulmonary vascular resistance Other symbols as in Fig 2

±S E M Tests of the statistical significance of differences between pre and postoperative findings as well as between hemodynamic observations at rest and during exercise postoperatively were made using Student s *t* test *P* values higher than 0 05 were not considered to be significant

RESULTS

Before operation 10 patients were considered to be in functional class IV 22 in class III and one patient in class II Subjective improvement was noted in 28/33 of the patients The condition improved three classes in 2 patients two classes in 9 patients (27%) one class in 17 patients (51%) and remained unchanged in 5 (15%) The latter 5 patients belonged to class III No patient in any class became worse A comparison between pre and postoperative functional classification is shown in Fig 1

Hemodynamic data from the pre and postoperative catheterization studies are presented in Figs 2 and 3 The oxygen uptake averaged 105% of the predicted normal value and there was no significant difference between pre and postoperative values There was a general tendency towards higher postoperative flow levels although the range of variations was very wide The mean cardiac index (CI) increased significantly from 2 18±0 58 (S D) to 3 00±1 01 l/min/m² after valve replacement A CI of 3 0 or more was found in about 1/3 of all patients Stroke index (SI) averaged 28 8±9 96 (S D) and increased by 12 6 ml to 41 4±13 38 ml/m² after surgery The increase in stroke volume was the main determinant of the increase in cardiac output There was a significant decrease in A–V O_2 difference from a mean value of 64 4±15 41 (S D) to 44 8±8 81 ml/l reflecting the higher cardiac output in relation to oxygen uptake A definite improvement in right heart pressures occurred The pulmonary artery mean pressure (P_{PA}) and pulmonary capillary venous (PCV) pressure both decreased significantly postoperatively The PCV pressure was reduced from a mean of 18 0±7 01 (S D) to a mean of 11 6±5 51 mmHg but remained above the normal range of 10 mmHg in nearly one half of all patients examined A significant reduc

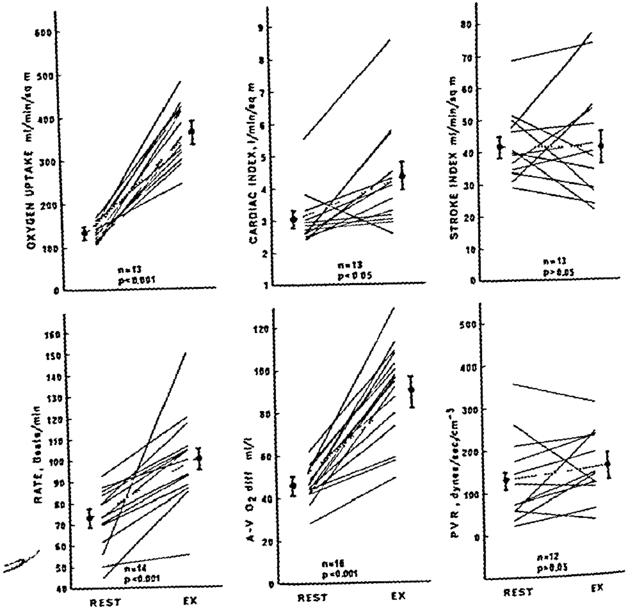


Fig. 4. Hemodynamic data at rest and during exercise after mitral valve replacement. Symbols as in Fig. 3.

tion occurred in the calculated pulmonary vascular resistance (P_{VR}). Right atrial mean pressure (P_{RA}) remained unchanged and was above the normal range in most patients.

The postoperative hemodynamics during exercise are presented in Figs. 4 and 5. As a precaution exercise testing was restricted to patients with normal or moderately elevated PCV pressure at rest. The mean CI increased significantly with exercise from a resting value of 3.00 ± 0.82 (S.D.) to 4.30 ± 1.57 l/min/m². The increment in cardiac output was predominantly due to an increase in heart rate and the small increase in SI was not significant. The response of cardiac output to exercise was inappropriate in relation to the oxygen uptake in all but 3 patients (Fig. 6). The abnormally low increase in cardiac output compared with the amount of exercise performed was associated with a widening of the A–V O_2-difference which increased from a mean of 46.5 ± 7.64 (S.D.) to 90.2 ± 21.12 ml/l. The mean PCV pressure increased with exercise from 10.3 ± 3.80 (S.D.) to 22.5 ± 6.05 mmHg exceeding 17 mmHg in all but one patient. Left ventricular end diastolic pressure increased significantly and con-

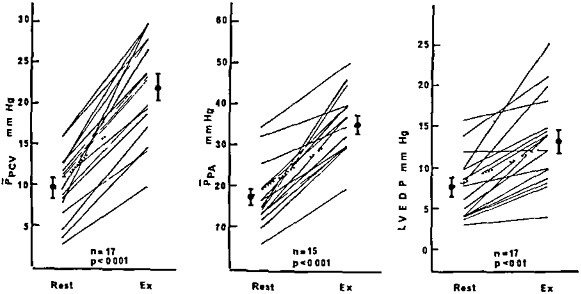


Fig 5 Pressure recordings at rest and during exercise 12–24 months after mitral valve replacement Symbols as in Fig 2 *LVEDP* = left ventricular end-diastolic pressure other abbreviations as in Fig 3

tributed to the rise in PCV pressure As expected some increase in P_{PA} occurred secondary to the increase in PCV pressure Most patients showed unchanged values for PVR indicating that the cardiac output response in general was sufficient to compensate for the abnormal rise in pulmonary artery pressure The average diastolic pressure gradient across the valve was 7 4 (range 2–18) mmHg at rest and increased to 12 7 (range 6–21) mmHg during exercise

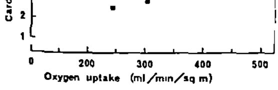


Fig 6 Cardiac output response during exercise in relation to oxygen uptake —=the regression line =the limits of normal values of cardiac output in relation to the oxygen uptake during exercise (5)

No close correlation was established between PCV pressure pulmonary artery pressure PVR or the diastolic gradient across the mitral valve at rest and during exercise and the patients functional status (Fig 7)

Total heart volume was enlarged in most patients A slight decrease from a preoperative mean of 1 264±414 1 (S D) to 1 155±418 5 ml after operation was found but the reduction in heart volume was statistically insignificant ($t=1\ 039$ $p>0\ 05$) A comparison between pre and postoperative heart volume is given in Fig 8 Fourteen patients had no change in heart size after mitral valve replacement (±10%) and four had a larger heart size postoperatively than before operation Therefore in 18/33 patients (55%) the heart failed to decrease or increase Decreases of more than 20% of the preoperative value were observed in 6/33 patients (18%)

DISCUSSION

The present study has demonstrated that replacement of the diseased mitral valve with the new Bjork–Shiley tilting disc valve prosthesis results in significant hemodynamic and clinical improvement Thus our report confirms the results of Bjork et al (4) that the effect on circulatory kinetics in their patients seemed to be more significant than in other groups of patients who have undergone surgical treatment for mitral valvular heart disease—the

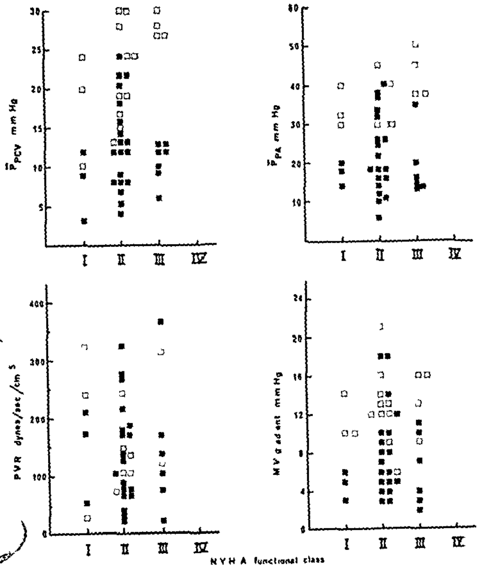


Fig 7 Correlation of postoperative hemodynamic variables with the clinical status 12–24 months after mitral valve replacement ■=at rest □=during exercise MV =mitral valve other abbreviations as in Fig 3

hemodynamic improvement was even more pronounced in our than in their study The improvement in cardiac output demonstrated here contrasts with most other reports on the results after mitral valve replacement Judson et al (8) reported no significant changes in cardiac output at rest and increases in response to exercise after mitral valve replacement with the Starr–Edwards prosthesis No significant increase at rest and an almost significant increase during exercise were observed in patients with Starr–Edwards and Cutter–Smeloff prostheses (10) Hultgren et al (7) observed a slight increase in cardiac output at rest and during exercise in patients with a Starr–Edwards prosthesis We found in a previous study (12) that the increase in cardiac output after replacement with the new Lillehei–Kaster pivoting disc valve prosthesis was insignificant compared with preoperative values In the present study as well as in other studies referred to above the cardiac output response to exercise in relation to oxygen uptake in general was reduced and was due almost exclusively to an increase in heart rate with unchanged stroke volume The reason for this impaired cardiac response to exercise is not fully understood but in addition to a varying degree of obstruction to the forward flow at the mitral valve during exercise it is probable that a myocardial factor may play a significant part (7)

Mitral valve replacement resulted in an efficient elimination of the filling resistance and/or insuffi

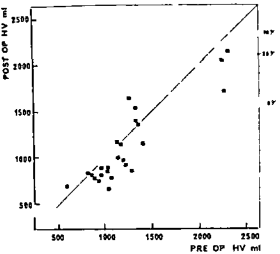


Fig 8 Total heart volume (*HV*) before and after mitral valve replacement

ciency of the diseased valve Postoperatively the resting pressures in the pulmonary circuit were relatively moderate and in general similar to or even lower than those usually noted after mitral valve surgery with other prosthetic devices (10 11 14 16) However with exercise abnormally high values for PCV pressure and pulmonary artery pressure were recorded in nearly all patients due partly to an increase in diastolic pressure gradient across the valve but also to a failing left ventricle

Almost all of our patients experienced dramatic symptomatic improvement Symptomatic relief after mitral valve replacement is related to a decrease in left atrial or PVC pressure and an increase in cardiac output (2 8 16) In the present study there was no close correlation between the height of postoperative PCV pressures PVR or cardiac output at rest and during exercise on the one hand and the symptomatic status of the patients The lack of correlation between clinical improvement and the hemodynamic results after mitral valve operation has also been noted by others (1 9 10 14) It is apparent that in most cases the patient s opinion of the extent of his improvement contrasts with the more modest hemodynamic gain The rehabilitation of patients with mitral valve prosthesis therefore seems to be burdened with a discrepancy between symptomatic improvement on the one hand and the cardiac status and total physical capacity on the other This points to the importance of hemodynamic data in the objective assessment of cardiac surgery

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A Validation of Cause-of-death Certification in 1 156 Deaths

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ABSTRACT Swedish twins have been followed for mortality since 1961, when the Swedish Twin Registry was formed During the years 1961–73 there were 1 290 deaths among twins born in 1901–25 In 1 156 cases the cause of death could be established from collected records and classified according to the 1965 revision of ICD Using the review of records as the standard rates of detection and confirmation relating to the death certificate diagnoses were calculated It is concluded that Swedish death certificate data are fairly valid for use in epidemiological studies and mortality statistics with regard to most cancer forms, cerebrovascular disease, ischemic heart disease bronchitis asthma and emphysema accidents and suicides but not for diabetes mellitus alcoholism mental diseases rheumatic heart diseases and other heart diseases However in selected clinical–epidemiological studies it is often necessary to collect all available documents prior to judging the cause of death

Data on death certificates are usually the only basis for epidemiological studies on mortality The reliability of mortality statistics is dependent on several factors but clinical examinations in combination with autopsies are probably our best instruments for accurately establishing cause of death (1 14 19 24) However in cases of death outside hospitals especially sudden deaths we often have to rely upon other information in certifying the cause of death Therefore in order to get an adequate evaluation of the accuracy of the cause of-death determination we must collect all available documents and protocols on every deceased person in a representative population sample and from these documents make our decision Such a validation has not been performed before in Sweden We thought it justified to evaluate mortality in Swedish twin pairs because mortality data have been carefully kept on them since 1961

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MATERIAL AND METHODS

The Swedish Twin Registry

The Swedish Twin Registry was set up during the years 1959–61 At that time it contained about 10 000 pairs and covered 95% of all living Swedish same sexed twins who were born in the country between 1886 and 1925 The compilation procedure and the demographic structure of the twin series have been described elsewhere (3) The Registry was set up primarily to study factors of etiologic importance for diseases of the respiratory and cardiovascular systems It has been used for several questionnaire surveys (4–9) as well as for clinical studies on subsamples (10 17 20 22) Another highly important line of the Registry s research program is the continuous mortality follow up which has been in progress since 1961 (10 11 12 13)

Mortality evaluation

The Twin Registry has been compared annually with the death registry for Sweden as a whole Since 1971 this matching has been done each month at the Central Bureau of Statistics making the death certificate available for verification Besides the cause of death the certificate contains the name of the certifying physician pathologist or forensic medical officer and whether or not the deceased twin had been treated in a hospital For twins born in 1901–25 hospital records autopsy protocols police protocols information from general practitioners and information from other pertinent sources could then be collected The underlying cause of death was established from all the records The evaluation of all the twin deaths

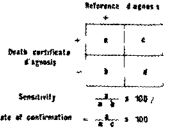


Fig 1 Definition of sensitivity and rate of confirmation

has been made primarily by one of us (U de F) When ever there was any form of uncertainty three physicians (U de F L F T L) made the final evaluation These evaluations were always performed without knowledge of the underlying cause of death as stated on the death certificate The underlying cause of death was classified according to a Nordic (Denmark Finland Norway and Sweden) adaptation for hospital use of the 1965 revision of the International Statistical Classification of Diseases Injuries and Causes of Death (ICD 1967) which has been used since 1969 in Sweden The Nordic edition differs from the 1965 version of the ICD mainly in its more detailed specification of subcategories denoted by a fifth digit (16)

Validation procedure

A validation measurement based on a comparison between our diagnosis and the diagnosis on the certificate is computed Our diagnoses have been considered the true judgement (reference diagnosis) The validity of the underlying cause of death as stated on the death certificate can then be described in terms of its sensitivity and the rate of confirmation as shown in Fig 1 The sensitivity indicates the ability of the death certificate to identify as many true positives (a+b) as possible whereas the rate of confirmation gives an estimate of how many death certificate diagnoses can be confirmed by the reference judgement A proper balance between these two measures of validity depends of course on both the design and the object of the study

RESULTS

Up to Dec 1973 1 290 twins born in 1901–25 had died In 1 156 cases the cause of death could be established from collected records but in the remaining 134 cases we had to rely on death certificates alone A death certificate as the only source of information on cause of death is relatively more common for accidents and suicides than for cancer of all sites (Table I) This is however understandable when one considers that most cancer patients usually die in hospital

Validation of death certificates has been performed in the 1 156 cases for which the cause of death could be established from collected documents such as hospital records etc The overall findings are summarized in Table II The causes of death have been grouped according to ICD numbers For cancer of all sites (ICD 140–209) a high sensitivity (98 2%) as well as a high rate of confirmation emerges (99 5%) This finding is consistent for all subgroups of cancer except leukemias (ICD 204–207) which show a sensitivity of 81 3% but a rate of confirmation of 100 0% For other neoplasms of lymphatic and hematopoietic tissue (ICD 200–203 208–209) the sensitivity was 88 9% and the rate of confirmation 92 3%

The detection of diabetes mellitus (ICD 250) on the death certificates as the true underlying cause of death is fairly low having a sensitivity of 60 0% Our mortality evaluation confirmed this disease as cause of death in 81 8% of the true cases The detection of alcoholism and mental diseases as true underlying causes of death is also relatively low with sensitivities of 81 0% and 59 1% whereas the confirmation rate was 94 4% and 100 0% respectively

The validity of the cause of-death determination in cases of ischemic heart disease (ICD 410–414) and cerebrovascular diseases (ICD 430–438) is on a fairly high level with sensitivities of 93 9% and 96 0% and rates of confirmation of 92 2% and 97 0% respectively Rheumatic heart diseases (ICD 393–398) are somewhat more difficult to detect through death certificates (sensitivity 85 3%) but nearly all of them are confirmed by our mortality evaluation (rate of confirmation 96 7%) The opposite trend is noted for other forms of heart disease (ICD 420–427) for which the sensitivity is 64 7% and the rate of confirmation as low as 36 7%

Table I *Relative distribution (%) of causes of death according to source of information*

ICD	Source of information	Death certificate	Records etc
140–209	Cancer all sites	18 6	33 7
340–458	Total disease of circulatory system	41 0	40 8
E95	Suicides	12 6	4 3
E80–E94 E96–E99	Accidents	13 4	4 4
Remaining ICD nos	Other diseases	14 4	17 3
Total no		134	1 156

Table II *The underlying cause of death coded from different types of information among 1156 deceased persons born in 1901–25*

ICD	Cause of death	Code no of: Record	Code no of: Death certificate	Agreements	Sensitivity (%)	Confirmation (%)	Autopsy (%)
140–209	Cancer of all sites	389	384	382	98.2	99.5	43.7
151	Cancer of stomach	41	40	39	95.1	97.5	41.5
152 153	*Cancer of intestine except rectum*	33	33	33	*100.0*	*100.0*	45.5
154	Cancer of rectum and rectosigmoid junction	12	12	12	100.0	100.0	16.7
155–156	Cancer of liver intrahepatic bile ducts gallbladder and bile ducts	11	11	11	100.0	100.0	54.5
157	Cancer of pancreas	25	26	25	100.0	96.2	48.0
162	Cancer of lung	43	44	43	100.0	97.7	48.8
170–173	Cancer of bone/skin	10	10	10	100.0	100.0	10.0
174	Cancer of breast	55	54	54	98.2	100.0	38.2
182	Cancer of uterus	22	23	22	*100.0*	95.7	40.9
183	*Cancer of ovary fallopian* tube and broad ligament	21	19	19	90.5	100.0	28.6
185	Cancer of prostate	17	17	17	*100.0*	100.0	35.3
188–189	Cancer of bladder and of other and unspecified urinary organs	11	11	11	100.0	100.0	54.5
191 192	Cancer of brain and of other parts of nervous system	14	14	14	100.0	100.0	50.0
190 193–199	Eye and other	18	17	17	94.4	100.0	44.4
204–207	Leukemia	16	13	13	81.3	100.0	43.8
200–203 208–209	Other neoplasms of lymphatic and hematopoietic tissue	27	26	24	88.9	92.3	74.1
250	Diabetes mellitus	15	11	9	60.0	81.8	26.7
291 303–304 571.00	Alcoholism	21	18	17	81.0	94.4	80.9
290–315	*Mental diseases*	*22*	*13*	*13*	*59.1*	*100.0*	*77.3*
340–349	Other diseases of central nervous system	19	18	18	94.7	100.0	31.6
390–458	*Total diseases of circulatory system*	*455*	*472*	*445*	*97.8*	*94.3*	*55.4*
393–398	Chronic rheumatic heart disease	34	30	29	85.3	96.7	52.9
400–404	Hypertensive disease	5	6	5	100.0	83.3	40.0
410–414	Ischemic heart disease	264	269	248	93.9	92.2	53.0
420–429	Other forms of heart disease	17	30	11	64.7	36.7	70.6
430–438	Cerebrovascular disease	100	99	96	96.0	97.0	53.0
441	Aortic aneurysm (non syphilitic)	7	6	6	85.7	100.0	85.7
450	Pulmonary embolism and infarction	19	24	17	89.5	70.8	84.2
480–486	Pneumonia	6	12	4	66.7	33.3	83.3
490–493	Bronchitis emphysema and asthma	22	22	22	100.0	100.0	77.3
531 533	Peptic ulcer	8	8	7	87.5	87.5	87.5
550–553	Intestinal obstruction and hernia	5	5	5	100.0	100.0	80.0
574–575	Cholelithiasis and cholecystitis	5	4	4	80.0	80.0	100.0
577	Diseases of pancreas	7	8	7	100.0	87.5	100.0
590–599	*Other diseases of urinary system*	24	25	24	100.0	96.0	62.5
710–715	Arthritis and spondylitis	6	5	4	66.7	80.0	50.0
780–795	Senility symptoms and other ill-defined conditions	7	3	1	14.3	33.3	57.1
E80–E94 E96–E99	Accidents	50	51	50	100.0	98.0	62.0
E95	Suicide	52	50	50	96.2	100.0	63.5
N80–N99	Injuries	19	14	12	63.2	85.7	78.9

Among respiratory diseases the group including bronchitis emphysema and asthma (ICD 490–493) is both detected and confirmed to 100% With regard to pneumonia (ICD 480–486) and pulmonary embolism and infarction (ICD 450) there is a tendency to overdiagnosis on the death certificate A thorough mortality evaluation often reveals other underlying causes of death in these cases This was most pronounced for pneumonias where a rate of confirmation of only 33 3% was obtained For pulmonary embolism and infarction the rate of confirmation was 70 8% The sensitivity was 66 7% and 89 5% respectively The misclassified cases of pneumonia and pulmonary embolism were evenly distributed among a host of diseases (cancer alcoholism etc) without apparent logic

Accidents and suicides are nearly always classified accurately For most other diseases there are not yet enough deaths to warrant further conclusions

DISCUSSION

As mentioned earlier clinical examinations combined with autopsies are probably our best measure of the reliability of mortality statistics (1 14 19 24) In 1968 the autopsy rate in Sweden was about 45% of all deaths while for the age span 1–64 years it amounted to about 50% (24) which corresponds fairly well with the figure of 53 9% in the present material with an age span of 36–71 years The autopsy rate in Sweden is relatively high although for purposes of mortality evaluation an autopsy rate of 53 9% may seem low at any rate the autopsy rate is steadily increasing (24)

The accuracy with which causes of death are reported on death certificates has been investigated in a number of studies (14 15 21 23) Studies of this kind based on selected hospital materials with fairly high autopsy rates reveal a considerable number of diagnostic errors However mistakes made in the book keeping can have contributed to this Overestimation of the importance of autopsy findings may also be suspected

In a recent study on 400 consecutive deaths at a hospital with an autopsy rate of 96% the causes of death put forth before autopsy were compared with those established by the same clinician after autopsy (2) The clinical diagnoses proved erroneous in 30% of the deaths While such errors were most pronounced for patients above 70 years of age they also occurred in 15% of the patients below the age of 70 despite clinical diagnoses which seemed fairly certain This clearly illustrates the need for autopsy in as many cases as possible

However when certifying causes of death physicians are often restricted to hospital examinations or sometimes only to other medical examinations without post mortems Thus to allow an adequate evaluation of the accuracy of cause-of-death determinations in general we had to collect all available information from which the cause of death was determined This procedure enabled us to validate the clinical judgement in all cases There are of course drawbacks with the method used Our clinical diagnoses have been established retrospectively which may have biased our results Hopefully this limitation is counterbalanced by our routine of evaluating mortality in a standardized way by the same team of doctors throughout the study

With few exceptions cancer forms were certified accurately on the death certificates Regardless of whether the terminal episode occurs in or out of hospital most patients have entered a hospital and undergone similar diagnostic procedures during the courses of their illness It is, however obvious that malignant diseases are sometimes overlooked clinically as judged from the autopsied cases so that statistics on cancer morbidity cannot be based on information from death certificates alone (18)

The frequency of diabetes mellitus alcoholism and mental disease as underlying causes of death is often underestimated clinically Diabetes mellitus is often recorded as a contributory rather than underlying cause of death (14) This is to some extent an illustration of the difficulty inherent in certifying the correct underlying cause of death when several chronic diseases exist at the same time Such situations are especially common in old people Cause-of-death statistics based on death certificate data are of course of minor importance in such cases (18) The low detection rate of mental diseases (59 1%) and to some extent of alcoholism (80%) may partly be attributed to the negative connotation with which these syndromes are still burdened not only by the general public but also by doctors

The accurate certification of ischemic heart disease as an underlying cause of death seems to be fairly valid Compared with pathologists clinicians are often inclined to diagnose more cases of ischemic heart disease in deaths in younger age

groups and fewer in the older (1 14) A slight over diagnosis was seen in the present study The relatively high accuracy of cause-of-death determination with respect to ischemic heart disease probably reflects the increasing knowledge of diseases Autopsy studies reveal a lot of myocardial infarctions which have been overlooked clinically (2 19) This seems to happen especially often in patients with chronic ischemic heart disease (2) but the underlying cause of death is still labelled as ischemic heart disease in many of these cases There are nonetheless reasons to believe that the real frequency of ischemic heart disease is higher than official mortality statistics show (19) Rheumatic heart diseases on the other hand are now rarely seen and when they do appear seem somewhat more difficult to detect than ischemic heart disease

Both pulmonary embolism and pneumonia have been subjects of considerable diagnostic difficulty with many false positive reports on the certificates This was especially true for pneumonia However a sensitivity of 89 5% for pulmonary embolism must be considered unexpectedly high The over diagnosis of pulmonary embolism is probably to some extent due to an overestimation of the importance of pulmonary emboli found at autopsy Bronchitis asthma and emphysema as the underlying *causes of death were both detected and con*firmed in every case This was almost as true for cerebrovascular diseases accidents and suicides For most other groups of diseases the numbers of deceased twins were too few to warrant study

On the basis of the present results it can be concluded that Swedish death certificate data are fairly valid for use in epidemiological studies and mortality statistics with regard to most cancers cerebrovascular disease ischemic heart disease bronchitis *asthma and emphysema accidents and* suicides but not for diabetes mellitus alcoholism mental diseases rheumatic heart diseases and other heart diseases However in selected clinical epidemiological studies all available documents should be collected in every case prior to judging the cause of death

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Five-year Mortality in the City of Bergen, Norway, According to Age, Sex and Blood Pressure

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ABSTRACT The City of Bergen was covered by a Mass Miniature Radiology Survey in 1963–64 On the initiative of the University of Bergen examinations of BP were included The initial survey has been reported previously (1) This analysis concerns the relationship between the 5½ year cause specific mortality and BP Non attenders have excess mortality in relation to attenders and this is mostly explained by a generally high mortality among bedridden people The age specific total mortality shows a clear pattern of a general increase with increasing BP At high systolic BP levels the 5 year mortality is independent of whether the age is 45 or 75 The systolic age adjusted curve for males increases quite linearly while the diastolic curve is more U shaped Thus when comparing the predictive power of BP allowance must be made for this fact Using a second order polynomial prediction function diastolic BP seems to be somewhat better than the corresponding systolic BP for age adjusted total mortality However using a linear prediction function this conclusion is reversed The mortality from cerebral stroke shows a dramatic increase with increasing BP The diastolic curve shows a bend-off for high values above 110 mmHg This may be due to the offer of treatment which such patients received after the screening Also the CHD mortality curve flattens for high BP values especially for diastolic BP

Several prospective as well as retrospective studies on mortality as a function of BP have been reported (3 7 8 9) These studies vary in size representativeness time of observation etc and form the basis for considerable knowledge on the topic

The following advantages have prompted us to perform the present study 1) The study covers a complete community a medium sized town on the western coast of Norway 2) Relative to similar follow up studies the size of the population is rather large (≈70000) 3) The study covers both sexes 4) The BP measurements were performed using a standardized technique by carefully trained personell (4 5 6) 5) The BP pattern among the non attenders was examined at home visits on a random 10% sample (1) 6) An automatic follow up procedure of deaths using a nation wide system of individual registration numbers secured an almost complete follow up coverage

MATERIAL AND METHODS

Population

The population in this study 41488 males and 50618 females is defined as the registered population of the City of Bergen born in 1948 or before as of Oct 1 1964

The population of Bergen underwent a compulsory mass X ray screening for tuberculosis in Aug 1963–Feb 1964 The element of compulsion made it possible to ask for certificates in case of non attendance Some had recently had their lungs examined radiologically and some their BP measured by others These results have not been included The coverages of the examinations are given in Table I

BP was measured in a 10% random sample of non attenders The results of these measurements have been presented earlier (1) The attendance varies by age and sex as shown in Table II Young adults (20–29 years) and people over 70 have low attendance Of particular interest is the low attendance for young males (sailors students soldiers) The reasons for non attendance are known for 73% of the non attenders and are summarized in Table III The most common reason for not attending was a recent X ray examination by others acceptable from a TB point of view but generally unfortunate since BP measurements were excluded

The examination was based upon individual letters of invitation Recent migrations therefore created occasional

Table I *Coverage (%) of examinations*

Population		X ray	BP
Males	41 488	89 9	69 6
Females	50 618	92 4	82 5
Total	*92 106*	*91 3*	*76 7*

problems in the identification of current addresses However the mortality has been followed for everybody irrespective of attendance or geographical location in Norway at the time of death

Deaths

All information about deaths in the follow up period is based solely on official death certificates available in the Central Bureau of Statistics Oslo The causes of death are coded according to the 7th and 8th revision of the WHO classification system Access to this central file was granted for the follow up purpose The codes are condensed to an aggregated list of 48 causes of death (12) In addition to the total mortality deaths from CHD and cerebral stroke will be studied in this paper The distributions of deaths by age and sex are given in Table IV Throughout this study only simplified 5 25 year death rates are used defined as the number of deaths during the follow up period (Oct 1 1964–Dec 31 1969) divided by the initial number of persons exposed to risk These rates should be quite adequate as long as they are low and the follow up period is short In the analysis of the findings age adjusted rates are also used The adjustment is done according to the indirect method (2)

Examinations

The BPs were measured according to a procedure de bed previously (3) The diastolic pressure was meas d in the 4th as well as in the 5th phase the latter being ed in the present report

RESULTS

Attendance

The 5 25 year death rates for attenders and non attenders are given in Fig 1 by age and sex An excess mortality for the non attenders is clearly demonstrated For young females the mortality for non attenders is 3–4 times that of the attenders for young males it is somewhat lower but still considerable The relatively higher excess mortality for non attending females compared with males might be related to the higher general attendance for females The reason for non attendance is available for 73 % of the non attenders Fig 2 shows that only the group of bedridden or sick persons shows markedly clear excess death rates relative to all non attenders Mortality among the age groups 40–70 is on an average 3 times higher than among all non attenders

Table III *Reasons for not attending BP measurements*

	Males	Females	Total
In bed/sick	342	977	1 319
Under TB control	317	198	515
X rayed recently by others	5 724	3 125	8 849
Other specified reasons	3 457	1 582	5 039
No reason	2 794	2 975	5 769
Total	12 634	8 857	21 491

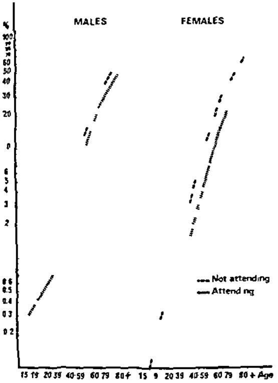
Blood pressure

Age and sex specific death rates are calculated according to systolic as well as diastolic BP (Figs 3–6) The age specific curves show a pattern of general increase in mortality by size of pressure a pattern which gradually disappears for higher age groups For males one might say that if the systolic BP is above 210 mmHg the 5 year mortality is independent of whether the age is 40–49 or 70–79 (Tables detailing the total material with respect to numbers of deaths rates etc are available in stencil form on request)

The relationship is given on a semilogarithmic scale and seems to be rather linear for most of the scale for the systolic pressure However the females have a very clearly U shaped function indi

Table II *Age and sex distribution of eligible population (%) covered by the BP measurement*

	Age (y) 15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–89	90–94	≥95
Males	65 4	48 2	62 1	65 4	68 9	69 4	72 3	74 8	76 5	76 9	81 9	86 5	81 6	74 6	68 0	46 4	33 3
Females	78 9	72 4	79 9	84 2	86 1	86 4	86 5	87 1	87 6	86 0	86 5	85 2	75 8	65 4	48 4	38 2	50 0

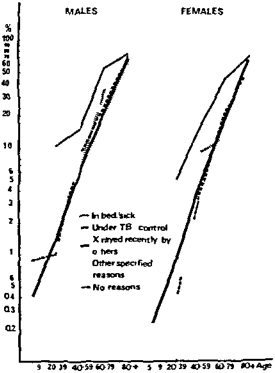


F g 1 Dea h rates (%) f om all causes by sex a e and a endance

cat ng an ncreased mortal ty for the extreme lo v le els as well This appl es to both sexes in the case of the d astol c pressure The d astol c pressure shows another dev at on from l nearity vith a bend-off at the h gher values A method for test ng whether or not the bend off is stat st cally s gn fi cant s described in Append x 1 Apply ng th s method o the data for the d astol c curve for males the bend-off is stat stically s gn f cant on level $p <$ 0 05 but not for females Th s and s m lar p values n th s paper should be nterpreted with care bear ng n m nd that they apply to hypotheses formu lated on the bas s of the present material

The age adjusted figures are given sex spec f cally for the two measurements in F g 7 For males there is a cont nuous ncrease in mortal ty from a systol c pressure of 100–110 to above 200 mmHg For females the curve s U shaped however the *fract on of the female populat on that is affected by* the increased left s de mortal ty is rather negligible (the number of dead females all ages th systol c pressure <120 is 86 of 2208 i e 3 9%) The ob served d fferences between systol c and d astol c pattern ind cate d fferences in their pred ct ve value as to mortal ty

The systol c curve for males ncreases quite l nearly whereas the d astol c curve i more U shaped When comparing d fferences betv een the tv o BP measurements v ith respect to pred ct ve po ver the d fferent shapes of the t o curves must be taken nto account Us ng only a l near pred c tion function (d scrim nant funct on) as is done by Kannel et al (8) gives the systol c BP greater pred ctab l ty purely through its l near risk funct on as compared with the d astol c Thus it may be that a curved d astol c BP funct on vill give qu te as *good predict ons with respect to death as a l near* systol c In Append x 2 three pred ct ve nd cators are proposed for the comparisons such as mult ple correlat on coeff cient percentage reduct on of S D and w dth of conf dence intervals of the rates at the mean BP As pred ction funct ons are used both f rst and second order polynom als of the age adjusted rates The ind cators enable us to compare the pred t ve power of the two types of BP The dth of the conf dence band at the mean BP s



F g 2 Dea h rates (%) from all causes by sex age and reason for not at end ng

Table IV *Number of deaths—total and specified for selected diagnoses—during 5.25 years*

	Age (y)										
Diagnoses	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69
Males											
Stroke			1	2		4	5	9	22	38	87
CHD	1			2	6	31	43	78	119	157	167
Other myocardial				1		2	1	2	4	14	28
Other cardiovascular		3			2	3	6	5	12	15	22
Sudden death		1			2	4	10	18	8	24	17
Hypertension							4	3	2	12	11
Total	16	20	19	27	38	85	139	217	325	462	567
Females											
Stroke					1	4	4	5	18	42	80
CHD					1	1	6	15	35	69	97
Other myocardial						2		1	1	8	29
Other cardiovascular	1	1			1	1	4	5	8	15	19
Sudden death				2	1	1	2	5	4	6	7
Hypertension						1	1	3	6	18	9
Total	4	5	4	14	23	44	75	120	192	334	477

probably the most efficient measure of the three for this comparison Table V*a* gives the sex specific indicators A linear systolic BP prediction function for males is obviously better than a linear diastolic However the opposite conclusion seems to hold for both sexes when using second order polynomials as a prediction function A systolic second order function gives over 50% greater width than a diastolic one for females and about 25% for males

A further observation from Fig 1 is that females

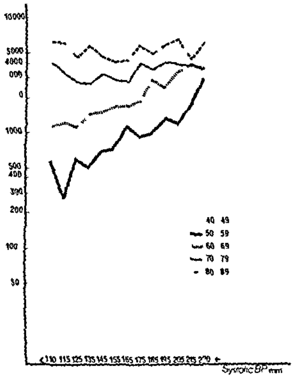


Fig 3 Age specific death rates per 10 000 from all causes by systolic BP Males

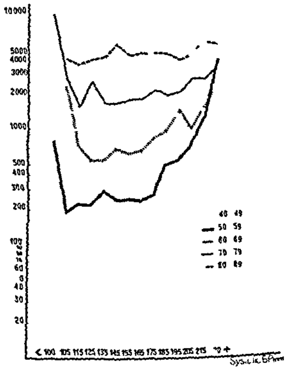


Fig 4 Age specific death rates per 10 000 from all causes by systolic BP Females

70–74	75–79	80–84	85–89	90–94	≥95	Total
83	79	33	30	2		395
179	99	48	9	2		891
44	49	40	23	2		210
17	16	12	4	2		114
15	15	5	1		1	116
8	9	3		3		53
574	442	290	134	20	4	3 318
93	113	111	51	9	3	534
110	88	63	30	7		517
55	75	70	53	21	4	319
17	25	22	24	4	1	148
8	4	5	4			49
19	17	17	6			97
522	606	494	297	80	13	3 254

Hereof 4<15 years

may have 30–50 mmHg higher systolic BP than males without a correspondingly higher death rate. For the diastolic pressure the difference is less striking (10–20 mmHg).

Cause specific mortality by age sex and blood pressure

Stroke. The age and sex specific stroke death rates are given in Figs. 8 and 9 by systolic as well as by diastolic BP. The correlation with BP is higher in younger persons than in the elderly. Men in their fifties who have a systolic BP of 200 mmHg have a mortality rate from stroke which is approximately 40 times higher than for those with systolic BP of 120 mmHg. On the other hand, when systolic BP exceeds 200 mmHg, *mortality from this cause seems to be independent of age*. The age adjusted stroke rates are also given in Fig. 10 by sex and BP. The rates seem to grow rather exponentially with increasing BP. An interesting deviation is the bend off for the male curve just like the case for the total mortality curve and again most markedly for the diastolic.

The same technique as above has been applied for testing the significance of the bend off for the age adjusted rates. For the diastolic male curve the bend-off is significant only with $p<0.15$ compared with $p<0.05$ for the systolic one. The reason why *the systolic bend off is more significant than the* diastolic is probably that only 2 cases of 357 pro-

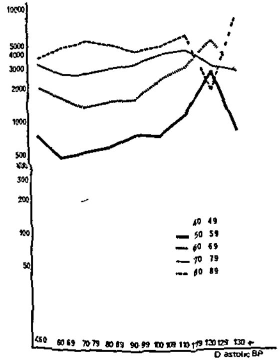


Fig. 5. Age specific death rates per 10 000 from all causes by diastolic BP. Males.

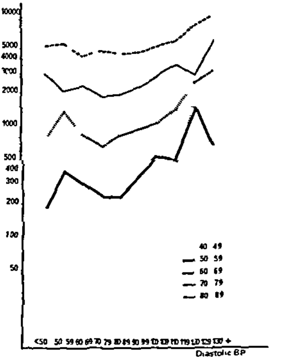


Fig. 6. Age specific death rates per 10 000 from all causes by diastolic BP. Females.

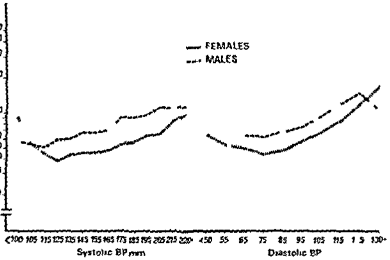


Fig 7 Age adjusted death rates per 10 000 from all causes by sex and BP

Table V *Comparison of predictive power between systolic and diastolic BP by cause and sex*
1st and 2nd order polynomials are used as prediction functions

	Males				Females			
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP	
	1st ord	2nd ord	1st ord	2nd ord	1st ord	2nd ord	1st ord	2nd ord
(a) All causes								
Multiple correlation coefficient	0 95	0 97	0 83	0 99	0 59	0 90	0 48	0 99
% reduction of standard deviation	65 9	72 7	39 1	83 6	15 2	52 8	5 3	83 2
Width of confidence band at the mean blood pressure	29 9	24 5	69 7	20 1	43 8	24 7	87 7	15 4
(b) Stroke								
Multiple correlation coefficient	0 83	0 96	0 78	0 95	0 76	0 84	0 77	0 98
% reduction of standard deviation	42 0	68 0	31 3	63 2	32 2	39 6	31 0	78 9
Width of confidence band at the mean blood pressure	11 5	6 5	31 6	18 3	13 9	12 6	22 6	7 2
(c) *CHD*								
Multiple correlation coefficient	0 95	0 95	0 74	0 94	0 87	0 87	0 80	0 98
% reduction of standard deviation	66 6	65 2	76 1	58 3	47 5	46 3	35 1	76 0
Width of confidence band at the mean blood pressure	11 5	12 2	29 2	17 8	12 0	17 5	14 1	5 5

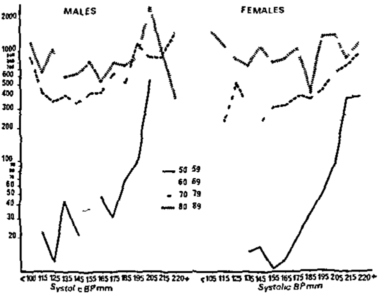


Fig 8 Age specific stroke death rates per 10 000 by sex and systolic BP

duced the latter compared with 26 cases of 1 016 for the former The predictive power of the BPs is again compared by the method described above (Table V*b*) The male systolic BP seems to give more precise predictions than the diastolic while the opposite conclusion seems to hold for females

Coronary heart disease Figs 11–12 give the CHD death rates by age sex and BP Again the age specific rates show a higher correlation with BP in the younger ages though not as dramatically as in the case of stroke Here too there is an almost age independent CHD mortality for persons with BP above 200 mmHg especially for men

The age adjusted rates given in Fig 13 demonstrate the basic log linear relationship between BP and mortality from CHD There is however a possible non linearity for the highest values This bend off effect agrees to some extent with other findings (7 10) where the curves stop increasing at the higher BP values Thus a reasonable null hypothesis is that the BP curves increase but approach asymptotic values A test method is described in

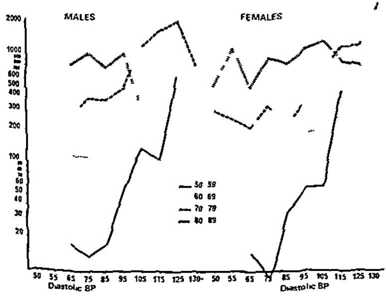


Fig 9 Age specific stroke death rates per 10 000 by sex and diastolic BP

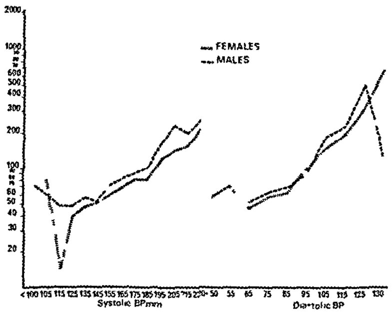


Fig 10 Age adjusted stroke death rates per 10 000 by sex and BP

Appendix 3 The hypothesis could not be rejected in any of the BP curves

The predictive power of the two BPs has been investigated as above and the results are given in Table Vc Again the male linear systolic predictor gives more precise predictions than any of the diastolic ones The second degree female diastolic function seems to give better predictions than any of the systolic ones

The excess CHD mortality for males in relation to females seems to explain most of the male total excess mortality It is striking that a female having diastolic BP of about 115 mmHg has an age adjusted mortality from CHD corresponding to males with a pressure of about 80 mmHg

DISCUSSION

The basis of this analysis is the information obtained from official death certificates The existence of discrepancies between clinically based records and post mortem examinations is well known (11) but it was not possible to revise the codes according to all information available from death certificates hospital records etc about the circumstances at death The autopsy rate in Bergen was about 30%

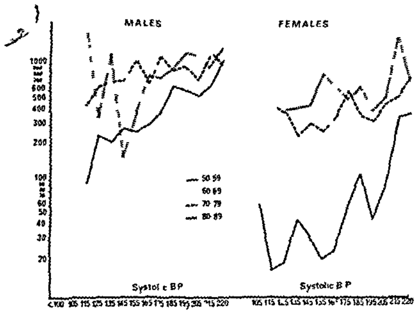


Fig 11 Age specific CHD death rates per 10 000 by sex and systolic BP

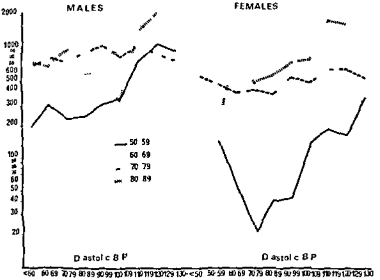


Fig 12 Age specific CHD death rates per 10 000 by sex and diastolic BP

for males and 25% for females at the time of investigation but may have been somewhat higher for deaths from CHD and cerebral stroke and for males of middle age Presumably codes were rather precise for about half of the deaths The remaining half vary in quality as to specification of cause of death Even if this varying quality leads to some bias in the total death rates from CHD and stroke it is nevertheless believed that the degree of association between BP and death from these two causes is rather unbiased However it may happen occasionally that a doctor's knowledge of observed hypertension has biased his conclusions as to the cause of death and it is impossible to estimate to what extent this may have been done

Both the systolic and the diastolic BP are significant risk factors for death in the City of Bergen The risk is usually U shaped with the low below the average BP Above this range the risk grows steadily and there is no sign of a threshold in full agreement with findings by others (8)

The rather age independent mortality rate for BPs above 200/120 mmHg holds true not only for the total mortality but also for the mortality from stroke and CHD These findings are to some extent in accordance with those from other extensive

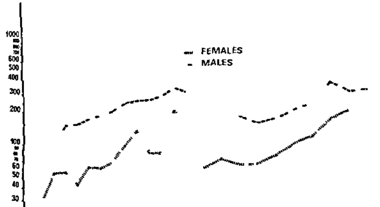


Fig 13 Age adjusted CHD death rates per 10 000 by sex and BP

BOOK REVIEW

Pediatrics 2nd ed Edited by Mohsen Zia with Charles A Janeway and Robert E Cooke 1021 pp Circ US$15 Little Brown and Company Boston 1975

This paperback textbook has as its main editor a dynamic *professor of pediatrics from Iran Of the 73 contributors* 20 or so represent countries outside the US including Africa Asia and Latin America One would have hoped to see more of child health and a greater slant towards problems in developing countries where pediatricians are scarce and health problems afflicting children enormous But Pediatrics is basically a US oriented textbook though interspersed here and there with sections on tropical childhood problems It has many virtues but also some flaws

The presentation covers pediatrics in a broad sense including a number of chapters written by pediatric subspecialists (in dermatology ophthalmology otorhinolaryngology etc) Wherever motivated surgical aspects have been incorporated in the presentation The abundant material has mostly been brought well up to date It is a testimony to the large bulk of knowledge in pediatrics today that the more than 1000 pages permit only a brief mention of many fields

The text is clear and ample use is made of subheadings and italics Duplications do occur here and there but are on the whole infrequent considering the large *number of contributors The strong hands of the editors* are also evident in the good conformity of the presentation References are given in reasonable number for most of the chapters though for the non initiated reader they perhaps suggest that practically only US journals and monographs are worth reading It is easy to point out quite a few chapters which deal in a very scholarly manner with their particular topics (e g chapters on Prenatal development Nutrition Infectious disease and *immunity in the child etc)*

The five Appendices at the end of the book covering a total of about 240 pages and comprising topics such as Differential diagnosis Emergencies trauma and poisoning and Drugs and dosages make a valuable adjunct to the general presentation

It is always difficult to strike the right balance between various fields but some disproportions must be criticized Nephrosis is given six pages but the infinitely more *common and therefore* important urinary tract infections are only allowed three and a half pages The Heart and the great vessels is given 45 pages (including 24 pages on Congenital heart disease alone) Table 7 6–11 on Inborn errors covers 11 pages whereas cerebral palsy is only given three quarters of a page and Avitaminosis has no special section of its own (rickets is described under the musculoskeletal system!)

A major flaw is the lack of a special chapter devoted to Feeding of infants and young children with a strong practical focus It is true that food and feeding habits vary but there is sufficient conformity to justify dwelling at some length on this topic of basic importance The very *modest reference made to breast feeding is striking* There is no echo of the change of outlook in the 1970s with renewed promotion of breast feeding for many important reasons—not only in the developing countries where breast feeding in poverty groups is vital but also in affluent societies Over and above parenthetic references here and there all that is given about breast feeding is one third of a page under the section Newborn infant In the Index terms such as solids beikost and *weaning foods are not even mentioned*

The illustrations are almost entirely in the form of drawings many of them quite good simple and instructive but others not so appropriate at times reminiscent of a textbook in biochemistry (Figs 7 1–4 9 1–4) The lack of photographic material is of course the price to be paid for keeping the production cost low

It is easy to criticize and hard to create Granting its objective to be mainly a traditional disease oriented textbook Pediatrics should be looked upon as a very valuable achievement besides being well within the financial means of any medical student or MD

Bo Vahlquist Uppsala Sweden

Acta Med Scand 200: 241–248, 1976

Acute Poisoning with Dextropropoxyphene

Clinical Symptoms and Plasma Concentrations

Arne Gustafson and Bengt Gustafsson

From the Intensive Care Unit, Department of Internal Medicine, University Hospital, Lund, and the Medical Department, Astra Lakemedel, Sodertalje, Sweden

ABSTRACT. Out of 14 cases of poisoning assumed to be due to dextropropoxyphene-containing drugs, propoxyphene and its main metabolite norpropoxyphene could be demonstrated in 11. The concentrations of the drugs were determined shortly after admission and then after 2, 4, 6 and 10 hours (in four cases also after 16 hours). The highest plasma concentration of propoxyphene, 0.74 $\mu g/ml$, was found in one case of fatal poisoning. Another patient with a plasma concentration of 0.51 $\mu g/ml$ showed signs of severe respiratory depression but survived after respirator therapy. In the patients with lower plasma concentrations the poisoning had a benign course. In most cases the plasma concentration of norpropoxyphene exceeded that of propoxyphene even in the first blood sample.

Dextropropoxyphene has been a widely used analgesic drug. In 1973, 90 000 000 tablets containing dextropropoxyphene were sold in Sweden (33) and in the USA more prescriptions for dextropropoxyphene have been dispensed in retail pharmacies than for any other drug (25). In Great Britain the National Health Service prescriptions for dextropropoxyphene were 2 500 000 in 1970 (21).

Acute poisoning with preparations containing dextropropoxyphene is relatively infrequent in relation to the extensive use of the drug. In a recent Swedish material poisoning with dextropropoxyphene amounted to 2% of the total number of intoxications (12). About 3% of poisonings admitted to the Medical Intensive Care Unit in Lund during 1973 and 1974 were caused by dextropropoxyphene.

Overdosage with dextropropoxyphene is, however, often followed by serious symptoms soon after ingestion of the drug and a high mortality has been reported. In one material dextropropoxyphene was involved in about 25% of the total number of deaths in cases of poisoning admitted for intensive care (12). Because of the short delay between ingestion and serious symptoms, the deaths in cases of fatal poisoning often occur outside hospitals (5, 15, 33, 38, 39).

In fatal poisonings dextropropoxyphene has often been ingested with other drugs, especially barbiturates and/or ethyl alcohol (5, 17, 18, 31, 33) and the role of dextropropoxyphene for the fatal outcome has sometimes been difficult to assess (17). Determinations of plasma concentrations of dextropropoxyphene during the course of an intoxication have seldom been performed. When done, only single values are given without reference to the time between blood sampling and ingestion of the drug. Forensic chemical analyses have shown large variations in tissue concentrations of propoxyphene (15, 33, 38, 40). No relation was found between the amount of drug taken and the tissue concentration, probably due to variations in the time between death and post mortem examination (33).

The aim of the present investigation was to do serial determinations of the plasma concentration of propoxyphene in cases of poisoning with dextropropoxyphene and to study the possible relation between the plasma concentration and the clinical symptoms. Plasma concentrations of salicylic acid, butenemal and ethyl alcohol were also determined.

MATERIAL

In 14 admissions to the Medical Intensive Care Unit between July 1974 and June 1975 available information indicated the ingestion of a drug containing dextropropo-

Table I *Age sex dose of dextropropoxyphene intake of alcohol time to first blood sample and results of gastric lavage in 11 cases of poisoning*

+=tablets or remnants of tablets in aspirate 0=no tablets or remnants of tablets −=no lavage performed

Case no	Age (y)	Sex	Drug and no of tablets	Dextro-propoxy phene dose (mg)	Alcohol intake	Hours between intake of tab-lets and first blood sample	Gastric lavage
1	21	♂	Doleron natt 9	585	Yes	4	0
2	19	♂	Doleron 8 (?)	520 (?)	Yes	8 (?)	+
3	19	♀	Doleron 20	1 300	No	6–9	−
4	28	♂	Doleron 10 (?)	650 (?)	No	1.5	+
5[a]	22	♂	Doleron natt 10 (?)	650 (?)	Yes	2 5	+
6	55	♂	Doleron 4	260	Yes	4 5	−
7	16	♀	Doleron 75 (?)	4 875 (?)	Yes	3	+
8	17	♀	Doleron natt 15	975	No	2 5	+
9	17	♂	Doleron natt 23	1 495	No	7	+
10	31	♂	Terginox 13	423	No	4	0
11	46	♀	Doleron natt 6	390	Yes	6	−

[a] Patient 1 second admission

poxyphene No dextropropoxyphene was found in plasma in three of these patients who were excluded Thus the material consisted of 11 cases of dextropropoxyphene poisoning in 10 patients (one patient was admitted twice) There were 6 men (mean age 28 5 range 17–55 years) and 4 women (mean age 24 5 range 16–46 years)

The drugs ingested (Table I) had the following composition *Doleron® dextropropoxyphene chloride* 65 acetyl salicylic acid 350 phenazone 150 diethylaminoetylphenothiazinecarboxyl 5 and caffeine 50 mg Doleron® natt the *same composition* as *Doleron but instead of caffeine* 50 mg butenemal (vinbarbital) Terginox® dextropropoxyphene chloride 32 5 phenprobamate 300 and pentobarbitone 50 mg The information on the number of tablets taken was received from relatives or from the patients In some cases the number was uncertain which is marked by (?) in Table I Three patients (nos 1 4 and 11) had also taken other sedative drugs in small quantities

METHODS

Blood sampling and chemical analyses

Blood samples (10 ml) were obtained on admission and then after 2 4 6 and 10 hours In four cases samples were also taken after 16 hours and in two cases further samples were drawn after 20 or 24 hours The time between intake of the tablets and the first blood sample is given in Table I Immediately after sampling the blood was centrifuged and the plasma was frozen for subsequent chemical analyses performed at the Toxicology Laboratories and the Department of Research and Development Astra Sodertalje and at the Department of Alcohol Research Karolinska Institute Stockholm The following analyses were performed

Dextropropoxyphene and norpropoxyphene Propoxyphene and norpropoxyphene in plasma were determined by a mass fragmentographic method using an LKB 2091 gas chromatograph mass spectrometer with a 9060 multiple ion detector The method (1) is based on the same principles as those exploited by Wolen et al (37) and by Sullivan et al (32) the same deutered substances were used as internal standards The accuracy of the method for propoxyphene was assessed by analysis of plasma samples containing varied known concentrations At a mean concentration of 23 ng/ml the relative S D of a single determination was 5% ($N=10$) and the mean deviation from the true value was 0 47 ng/ml At 186 ng/ml the corresponding values were 1% ($N=10$) and 0 03 ng/ml The detection limit was 3 6 ng/ml At a mean level of 15 ng/ml of norpropoxyphene the relative S D of a single determination was 6% ($N=10$) with a mean deviation from the true value of 1 2 ng/ml At 122 ng/ml the values were 4% ($N=10$) and 0 03 ng/ml The detection limit was 2 6 ng/ml

Acetylsalicylic+salicylic *acid* The combined acetyl salicylic and salicylic acid was determined in plasma by a spectrofluorimetric technique (13)

Butenemal Butenemal was determined by a modified gas chromatography method (13) based upon a method developed by Ehrsson (6)

Ethyl alcohol *Blood* samples were taken on admission and after 2 and 6 hours The alcohol concentration was determined by an automatic ultra microdestillation method (3)

Clinical examinations

Routine laboratory examinations including venous standard bicarbonate and base excess were performed in all patients When indicated arterial blood was taken for measurement of pH P_{CO_2} standard bicarbonate and P_{O_2}

ECG was recorded on admission and on the following day The cardiac rhythm was monitored continuously

Blood pressure pulse respiratory rate and level of consciousness were estimated half hourly

Gastric aspiration and lavage were performed immediately after admission in all but three cases In six

Table II *Some clinical findings on admission*
0=awake +=drowsy ++=comatose

Case no	Degree of alertness	Respiratory rate (breaths/min)	Heart rate (beats/min)	Systolic/diastolic BP (mmHg)	QRS duration (sec)	Base excess
1	++	20	110	80/60	0 08	− 4 0
2	0	14	70	125/80	0 08	− 0 5
3	+	14	90	120/80	0 11	− 4 0
4	++	16	125	130/80	0 08	+ 2 5
5	++	0	VF	0	0 14[b]	−13 5
6	0	20	100	120/80	0 08	+ 2 5
7	++	0	115	100/?	0 12	−17 0
8	+	16	120	110/90	0 08	− 1 5
9	++	14	65	100/70	0 12	+ 1 0
10	++	9	65	110/90	0 08	− 5 5
11	0	15	85	150/100	0 08	− 1 5

[a] Ventricular fibrillation [b] after ventricular defibrillation

cases tablets or remnants of tablets were identified in the aspirate (Table I) Activated charcoal was given after the lavage

Psychiatric consultation was performed in all patients before discharge

RESULTS

Clinical findings and course of the poisoning

Table II shows the relevant clinical findings on admission in the 11 cases of poisoning Only case 5 deteriorated after admission the other patients improved in the subsequent course

Six cases were in deep *coma* on admission four of them had taken drugs containing barbiturate and one (no 4) a phenothiazine preparation Only one of these patients (no 7) had taken tablets containing dextropropoxyphene without barbiturate and no other sedative drug

The *respiratory rate* was 15 breaths/min or less in seven cases Two patients had apnoea on admission and required respirator therapy

Heart and circulation One patient (no 5) had circulatory arrest (ventricular fibrillation) on admission Sinus tachycardia was noted on admission in five cases in the other cases the *heart rate* was within normal limits No other arrhythmias were observed

On the first admission one patient (no 1) had a *systolic BP* of 80 mmHg and on the second admission the same patient (case 5) had circulatory arrest In the other cases the systolic BP was 100 mmHg or higher

ECG showed a widening of the QRS complexes in four patients (nos 3 5 7 and 9) In case 9 a right bundle branch block was recorded which remained even at discharge 33 hours after admission The QRS duration in this patient was 0 12 on admission and 0 10 at discharge

No patient showed signs of *pulmonary edema* *Convulsions* were seen only in case 7 *Metabolic acidosis* with base excess values less than −3 was observed in four patients The acidosis was grave in two patients (nos 5 and 7) who had apnoea on admission

In summary the poisoning had a relatively benign course in all but two patients who are reported in detail

Case 5 A 21 year-old man was first admitted in July 1974 after the ingestion of 9 tablets of Doleron natt and ethyl alcohol (case 1) He was comatose on admission but recovered consciousness after four hours There were no signs of respiratory depression The course was without complications and the patient was discharged after 20 hours

He was admitted again at 0 20 a m on March 31 1975 (case 5) According to his relatives the patient had ingested 15 cl ethyl alcohol at 3 00 p m on March 30 and at 10 00 p m he had taken probably 10 tablets of Doleron natt When the ambulance arrived at his home at 0 00 a m the patient was not breathing Artificial ventilation and external cardiac massage were started and continued during the journey to hospital On admission he was pulseless and apnoeic The pupils were dilated ECG showed ventricular fibrillation After ventricular defibrillation intracardiac injection of adrenaline and i v isoprenaline drip sinus rhythm occurred The QRS complexes were widened (0 14 sec) but after one hour ECG showed sinus rhythm with normal QRS complexes Respirator treatment was started on admission and continued After two

Table III *Maximum plasma or serum concentrations at chemical analysis*

Case no	Propoxyphene (µg/ml)	Norpropoxy phene (µg/ml)	Acetylsalicylic acid + salicylic acid (µg/ml)	Butenemol (µg/ml)	Ethyl alcohol (‰)
1	0 17	0 30	69	4 0	1 8
2	0 06	0 05	19	<0 5	–
3	0 47	0 78	385	<0 5	–
4	0 07	0 14	91	<0 5	0
5	0 74	0 39	133	10 2	2 0
6	0 04	0 21	23	<0 5	1 5
7	0 51	0 79	154	<0 5	0
8	0 27	0 66	148	19 0	0
9	0 23	0 35	190	14 0	0
10	0 06	0 14	110	<0 5	0
11	0 05	0 09	37	2 0	1 6

hours there was a tendency to spontaneous but insufficient respiration The pupils remained dilated and a metaraminol drip was required to maintain the systolic BP at about 100 mmHg Cardiac arrest (asystole) occurred 15 hours after admission

At autopsy the pathological changes were confined to the lungs and the heart Microscopical examination showed signs of bronchopneumonia and anoxic changes in the heart muscle Chemical analysis showed the following concentrations of drugs in the liver phenazone 1 9 barbiturate 0 8 and salicylic acid 1 4 mg/100 ml no propoxyphene

Case 7 A 16 year-old girl had had problems of adaptation at school during the last months before admission During the evening on March 19 1975 she ingested some alcohol and after a dispute with her parents she took about 75 tablets of Doleron at 4 45 a m on March 20 She was admitted one hour later During the journey to hospital she suffered a grand mal seizure On arrival at the emergency room at 5 45 a m she was comatose with low and shallow respirations followed by apnoea Systolic BP was 100 mmHg ECG showed sinus tachycardia widened QRS complexes (0 12 sec) with prominent S waves in all leads except lead III Endotracheal intubation was immediately performed and respirator treatment was started Twenty minutes after admission she had another grand mal seizure which immediately responded to 10 mg diazepam i v During the following hour she had twitching movements in arms and legs

One hour after admission the patient recovered consciousness Even though she showed spontaneous breathing when tested she tolerated the respirator treatment without needing any sedative drug So the respirator treatment was continued for safety until seven hours after admission Her spontaneous breathing was then sufficient and the blood gases were normal Repeat ECG six hours after admission showed normal QRS complexes The subsequent course was uneventful and the patient was discharged on March 21

Psychiatric consultation revealed that in none of the surviving patients could the overdosing of tablets be classified as a suicidal attempt in most cases as an impulsive act In three patients the history revealed an earlier episode of self poisoning In four cases the tablets used had been prescribed for another member of the family

Chemical analyses

Table III shows the results of the chemical analyses All values represent the maximum plasma or blood concentrations of the different substances during the period of blood sampling

The plasma concentrations of *propoxyphene* showed a wide range between cases as could be anticipated from the different numbers of tablets taken and the differences in time which had passed between ingestion and blood sampling In all but two patients (nos 1 and 10) the maximum concentration was found in the first blood sample in the other two patients it appeared in the sample taken two hours after admission The highest value was found in case 5 On the first admission (case 1) the same patient had a plasma concentration which was much lower even though the dose of dextropropoxyphene was said to be of the same order at the two incidents of poisoning A suspicion of a larger dose on the second admission is supported by higher plasma concentrations of salicylate and barbiturate The second highest value was found in case 7 with the largest ingested dose of dextropropoxyphene

The plasma concentration of *norpropoxyphene* was highest in the admission sample in seven cases In four cases the maximum concentration was reached in the second sample The plasma level of norpropoxyphene was higher than that of propoxyphene in the admission sample in all cases except nos 2 and 5

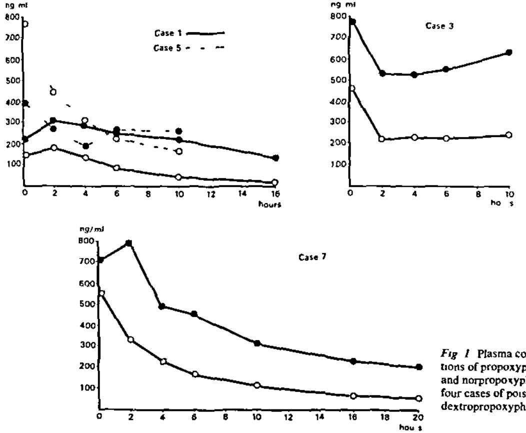


Fig 1 Plasma concentrations of propoxyphene (O) and norpropoxyphene (●) in four cases of poisoning with dextropropoxyphene

Fig 1 shows the plasma concentrations of propoxyphene and norpropoxyphene in relation to time after admission in four cases In cases 1 3 and 7 the norpropoxyphene concentration was higher than that of propoxyphene in all blood samples In case 5 the norpropoxyphene concentration did not exceed that of propoxyphene until six hours after admission and then the difference was only slight In the other three cases the quotient norpropoxyphene/propoxyphene was less than 2 in only 4 of 17 samples

All cases had measurable plasma concentrations of *acetylsalicylic and salicylic acids* The salicylate poisoning as such would be considered as moderate in case 5 with a plasma level of 385 μg/ml in the other cases the plasma concentration was lower

Measurable plasma concentrations of *butenemal* were found in all patients who had taken Doleron natt The highest plasma level was found in case 8 2 5 hours after ingestion of 15 tablets The overdose of barbiturate per se must be considered as slight to moderate as no patient had a plasma concentration above 20 μg/ml

Four patients had laboratory signs of a moderate poisoning with *ethyl alcohol* the blood concentrations were 1 5–2 0‰ In case 7 no ethyl alcohol was found in the blood though there was a statement of ingestion of alcohol

Course of the poisoning in relation to plasma concentrations of propoxyphene

There was a correlation between the plasma concentration of propoxyphene and the clinical course in that the highest plasma concentration was found in the poisoning with fatal outcome The patient with the second highest value exceeding 0 50 μg/ml also had serious symptoms Case 3 with the third highest value (0 47 μg/ml) had no life threatening symptoms In the other patients with a propoxyphene concentration on admission below 0 30 μg/ml the poisoning also had a benign course Case 10 had a low respiratory rate (9/min) for a short period The propoxyphene concentration was low but the drug ingested also contained barbiturate and phenprobamate

ECG changes with a widening of the Q[illegible]

plexes were seen in four patients Three of them had propoxyphene concentrations of above 0 40 μg/ml the fourth a plasma concentration of 0 23 μg/ml The role of propoxyphene in the latter case is difficult to assess since ECG showed a bundle branch block even after the other symptoms of poisoning had disappeared

DISCUSSION

Clinical symptoms

The clinical symptoms in incidents of dextropropoxyphene poisoning were described in the early 1960 s (14 23) and have been confirmed in subsequent reports (4 12 17) The course of the poisoning is characterized by signs of respiratory depression and often convulsions soon after ingestion of the drug Cardiac arrest may occur secondary to respiratory depression and apnoea Profound respiratory depression was also judged to be the primary cause of death in studies of the acute toxicity in laboratory animals (7) ECG changes with widening of the QRS complexes (12 26 30) and ventricular bigeminy (23) have been observed In surviving patients serious symptoms were of relatively short duration (2 4 26 40) even though recurrence of apnoea after initial improvement has been reported (9 10 19 29) The course of fatal poisoning is sometimes prolonged with symptoms secondary to cerebral anoxia (12 17)

It was pointed out that survival in acute dextropoxyphene poisoning may depend in part upon rapidity of absorption of the drugs but probably more important upon the timing and efficacy of resuscitative measures (17 19) This statement is illustrated by the two cases of serious poisoning in the present series In case 5 apnoea and ventricular fibrillation occurred at a time when the resuscitative treatment could not be instituted immediately resulting in cerebral anoxia and death In case 7 ventilatory assistance was started as soon as the patient became apnoeaic and she survived

Plasma and tissue concentrations of propoxyphene and norpropoxyphene

Plasma and tissue concentrations of propoxyphene have been studied in animal experiments after oral administration In the rat the concentration in plasma was of the same order as in the brain and the concentration of propoxyphene in the brain corresponded to the intensity of response and the duration of action of the drug (8) In studies of the acute toxicity in dogs convulsions were confined to the 15–20 min coinciding with a peak plasma concentration of about 1 4 μg/ml and the signs of toxicity subsided after six hours when the plasma concentration had declined to about 0 4 μg/ml (7)

The plasma concentrations of propoxyphene have been studied in humans after single oral doses up to 195 mg propoxyphene hydrochloride (13 27 28 35 36 37) The peak plasma concentration of propoxyphene was reached about two hours after administration (13 28 35 37) and the biologic half life was 11 8 hours (37) Propoxyphene is metabolized by N demethylation in the liver (18) the only major metabolite in humans is norpropoxyphene (24) Norpropoxyphene reached its peak plasma concentration 30 min later than propoxyphene The apparent half life for norpropoxyphene was 36 6 hours (37) There was an essentially linear relation between the dose and the plasma concentration of propoxyphene (35) but the concentration after equal doses showed a large variation between subjects (28 35) The mean peak concentration after 195 mg propoxyphene hydrochloride was 0 21 μg/ml and after 65 mg 0 06 μg/ml (13 35)

The propoxyphene concentration in blood and other tissues has shown a rather large variation in fatal human poisonings (15) However data are scanty when samples were taken for analysis in relation to the ingestion of the drug The blood concentration at autopsy was between 1 0 and 60 μg/ml in 238 cases of poisoning where propoxyphene was involved (6) In cases of fatal poisoning with propoxyphene only or in combination with alcohol the range of blood concentrations post mortem was 0 5–24 8 μg/ml (20 31) In 27 cases the mean concentrations of propoxyphene in blood and brain were 11 and 20 μg/ml respectively (15)

In two reports of propoxyphene poisoning the time between ingestion of tablets and blood sampling obviously was 1–2 hours in one fatal case after 2300 mg the plasma concentration was 0 97 μg/ml (17) and in another patient who had taken 700 mg and survived after a period of apnoea it was 0 90 μg/ml (40) These values are of special interest with respect to the present series

A blood concentration of 2 μg/ml has been suggested as the lowest level for establishing propoxyphene as the cause of fatal poisoning (22) However the plasma concentrations were lower (0 74

and 0 51 μg/ml) in the two cases of severe poisoning in the present study These values could be supposed to represent peak plasma concentrations since the blood samples were taken 2 5–3 hours after ingestion of the tablets The patient with the lower plasma level had taken a large dose but presumably the gastric aspiration recovered much of the ingested drug In the case with the highest plasma level the poisoning had a fatal outcome An additive effect of the simultaneously ingested barbiturate and alcohol must be taken in consideration In two studies (15 20) the mean blood concentration in fatal poisoning with propoxyphene only was higher than in lethal poisoning with propoxyphene and alcohol One of our patients had a plasma concentration of 0 47 μg/ml without serious symptoms The blood sample from this patient was taken at least six hours after ingestion of 1 300 mg dextropropoxyphene The absence of serious symptoms is difficult to explain but the fact that she had taken the tablets in divided doses over three hours might be of importance

The plasma concentration of *norpropoxyphene* was higher than that of propoxyphene even in the first blood sample in all but two patients of the present series In case 5 with fatal outcome the relation between plasma concentrations of propoxyphene and norpropoxyphene showed a different pattern from the other patients The time when the norpropoxyphene concentration exceeded that of propoxyphene was delayed to 6 hours after ingestion (Fig 1) A hypoxic liver injury from the cardiac arrest would be the reason for delayed metabolism of propoxyphene in this patient

It is not known whether norpropoxyphene has any pharmacological activity Considering the relatively short duration of symptoms in dextropropoxyphene poisoning and the long half life of norpropoxyphene its importance for the outcome of the intoxication may be questioned

The narcotic antagonists nalorphine and naloxene have been reported to reverse the respiratory depression after dextropropoxyphene overdosage (11 19) However they are not always effective (17) and in some poisoning centers (12 16) respirator therapy is considered to be safer than these drugs which were not used in the present study

In the present series of dextropropoxyphene intoxication six patients were less than 25 years old and the poisoning was in most cases characterized by the psychiatrist as an impulsive act This highlights the risk of prescribing this potentially hazardous drug especially in combination with barbiturates

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Therapeutic Implications of Renal Transplantation in a Patient with Fabry's Disease

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ABSTRACT In a patient with Fabry's disease who had undergone kidney transplantation to correct uremia, the neutral glycosphingolipids and α galactosidase activity have been measured in plasma and urine and, 9 months later, after the death of the patient in autopsy material After transplantation there was no significant increase in α galactosidase activity in plasma, the activity found never exceeded 3% of the mean control value A striking parallelism was found during the follow up period in the increase and decrease of trihexosylceramide and globoside and also of glucosylceramide and dihexosylceramide The α galactosidase activity in spleen and liver was as low as that observed in untreated Fabry hemizygotes These data and those obtained from autopsy material provide evidence that renal transplantation does not lead to a specific enzymic breakdown of trihexosylceramide in Fabry patients However, no trihexosylceramide accumulation was observed in the transplanted kidney

Fabry s disease an X linked metabolic disorder of glycosphingolipid metabolism is caused by a deficiency of a lysosomal α galactosidase ceramide trihexosidase (11) Although at least two α galactosidase isoenzymes known as α galactosidase A and α galactosidase B are present in normal tissue only the former isoenzyme is absent in Fabry s disease (23) The B isoenzyme (5–20% of the total α galactosidase activity) has only limited reactivity with trihexosylceramide (GL 3) In this paper α galactosidase refers to the A isoenzyme The abbreviations used are GL 1 (glucosylceramide) GL 2 (lactosylceramide) GL 2b (galactosyl-(α 1→4)-galactosylceramide) GL 3 (galactosyl (α 1→4) galactosyl-(β 1→4) glucosylceramide) GL-4 (N acetyl galactosaminyl (β 1→3) galactosyl (α 1→4) galactosyl-(β 1– 4) glucosylceramide)

The enzymic defect results in progressive accumulation of GL 3 in most tissues of the body especially in kidney (13 21) and the cardiovascular system (3 13) and also in liver (13 21 23) spleen (13 21 23) pancreas (21) and ganglion cells of the peripheral nervous system (21) Increased concentrations of GL 3 are also present in urinary sediment (8 23) and plasma (3 24) A second glycosphingolipid GL 2b is found in abnormally high concentrations in kidney (13 23) urinary sediment (8 23) and pancreas (21) For recent reviews see Kint et al (12) and Sweeley et al (23) Increased concentrations of GL 3 are found in all tissue sources analysed except erythrocytes in which catabolism of GL-4 the direct precursor of GL 3 does not occur

For several reasons Fabry s disease has been considered to be especially amenable to enzyme replacement therapy First of all it is a lysosomal storage disease Secondly the disease has a slow progression suggesting that even small amounts of substituted enzyme might be sufficient to cause degradation of excess amounts of glycosphingolipid in the various tissues Moreover the possibility exists that the enhanced plasma level of GL 3 is a major factor contributing to the accumulation of lipid in various tissues e g the cardiovascular system so that decreasing the plasma level might

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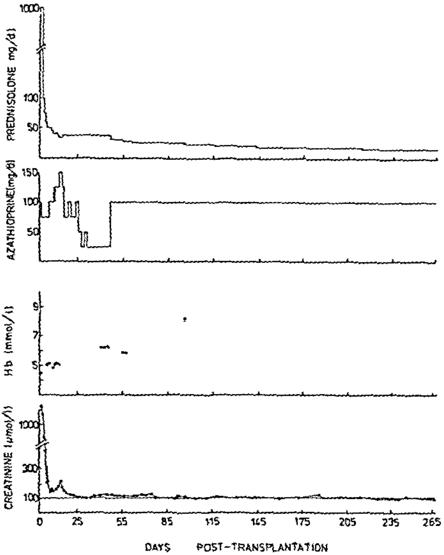


Fig 1 Prednisolone and azathioprine administered after transplantation and Hb and creatinine content in plasma after renal transplantation

slow down the accumulation process A fourth reason for enzyme substitution in Fabry s disease is that (as in the adult type of M Gaucher (10)) there is no involvement of the central nervous system For a recent review on enzyme replacement therapy see Rietra et al (18)

In recent years attention has been paid to renal transplantation in Fabry s disease as a means of introducing a continuous source of active enzyme capable of degrading accumulated material Since the healthy kidney contains the α galactosidase it seemed possible that degradation of circulating glycosphingolipid might occur either in the healthy kidney itself or indirectly by enzyme supplied by the kidney to the lysosomal system of other tissues (15) It should be stressed that most patients suffer from severe renal failure especially after the third or fourth decade so that the allograft eliminates the chronic uremia in such patients

There are controversial reports on the therapeutic effects of the transplanted kidney in providing enzyme and in bringing about a corresponding specific degradation of GL 3 in plasma (4 6 7 14 15 16) Philippart et al (14 15 16) and Desnick et al (6 7) have carried out successful transplantations on Fabry patients These investigators reported several clinical and biochemical improvements in the patients including a gradual decrease in plasma GL 3 The results of Clarke et al (4) and Wolfe et al (26), however suggest that

the decline in plasma GL 3 in Fabry patients following kidney transplantation may be due to alterations in the half life of erythrocytes the main source of this glycosphingolipid in plasma rather than to a specific enzymic action as claimed by the former authors

A Fabry patient who had been treated by regular hemodialysis for 5 years was admitted to our hospital in Aug 1974 and received a kidney trans plantation In view of the findings mentioned above the levels of a α galactosidase and glycosphingolipids were measured in plasma before and after transplantation and in autopsy material 9 months after the transplantation

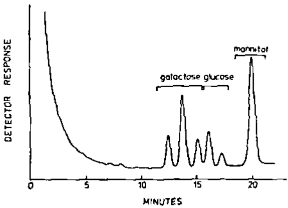


Fig 2 Gas liquid chromatogram of the trimethylsilyl derivatives of mannitol and of methyl glycosides from trihexosylceramide (GL 3)

CASE REPORT

A 40-year-old man the captain of a coaster was ad mitted to our hospital in Nov 1968 because of renal failure He had suffered from angina tonsillaris in 1963 and one year later in 1964 an asymptomatic proteinuria had been detected At that time his plasma creatinine was normal but had risen to 245 μmol/l in 1966 His kidney function deteriorated gradually He felt fairly well and worked normally until 3 weeks before admission when he complained of nausea vomiting and diarrhea In the past he had never experienced episodes of burning sensations in hands or feet During sea voyages to the tropics he never felt any discomfort from the heat and seemed to perspire normally

Physical examination disclosed a well nourished slightly uremic man with a BP of 180/100 mmHg and a regular pulse rate of 88/min Numerous purplish angi omatous lesions were visible predominantly on the lower abdomen buttocks thighs and scrotal area The heart was slightly enlarged and there was a pitting edema of the lower extremities Urinalysis revealed a proteinuria of 1 5 g/24 h and a low specific gravity of 1 005 Creatinine clearance was 2 5 ml/min urea 40 mmol/l and Hb level 5 3 mmol/l The AST was normal An ECG showed a severe left ventricular hypertrophy with very pronounced negative T waves The clinical diagnosis of chronic glomerulonephritis was made

Regular hemodialysis was started shortly afterwards using an arteriovenous fistula Bilateral nephrectomy was carried out in 1969 Microscopic examination of the kid neys revealed numerous lipid-containing vacuoles in tubular epithelial cells glomerular tufts and vessel walls The tentative diagnosis of Fabry s disease (angiokeratoma corporis diffusum) was confirmed by examination of skin biopsy material and by α galactosidase assays in plasma It was discovered later that the patient was a member of an extensive Fabry family

After nephrectomy the Hb level dropped to 3 5 mmol/l However transfusions of erythrocytes were rarely neces sary No erythrocyte half life determinations were per formed

Although the patient s BP remained normal during the five years of regular hemodialysis his ECG showed progressive signs of left ventricular hypertrophy

In Aug 1974 a cadaveric renal allograft was trans planted Data and treatment during the posttransplanta tion period are given in Fig 1 During the operation 3 U of packed cells were administered Graft rejection was suppressed by daily treatment with prednisolone and azathioprine The transplanted kidney started to function immediately and no dialysis was subsequently needed The postoperative course was complicated by leakage of urine from the anastomosis of the ureter into the bladder needing reimplantation of the donor ureter Because of re current urinary tract infections with several micro-organisms (E coli entercocci) the patient required anti microbial treatment (ampicillin nitrofurantoin) for several months No rejection crisis became manifest

At discharge in Sept 1974 the plasma creatinine was 105 μmol/l and the creatinine clearance 70 ml/min No proteinuria could be detected The Hb level gradually rose to 9 0 mmol/l Physical examination and chest X ray still showed an enlarged heart ECG did not change after transplantation Several months later the patient developed atrial fibrillation with a slow ventricular rate of 68/min for which no treatment was given

The patient died suddenly eight months after trans plantation presumably because of an arrhythmia Autopsy showed markedly enlarged organs with the following weights heart 820 g liver 2050 g spleen 480 g Lipid staining showed extensive deposits in the myocardium and endothelial cells of several organs (aorta lungs skin) and in muscle cells No abnormal lipid deposition could be detected in any of the histological sections of the transplanted kidney The ureter renal artery and vein were similarly free from lipid deposits Other clinical and pathological aspects will be described in detail elsewhere

METHODS AND MATERIALS

Enzyme assays

Fresh serum and heparinized plasma were assayed for α galactosidase activity using the artificial fluorogenic

Table I *α-galactosidase activity in plasma and serum of the patient and controls*

Days after transplantation	Activity (nmoles/h × ml)	
	Serum	Plasma
−133	0 33	
+1	0 63	
+6	0 54	
+12	0 42	
+84		0 58
+229		0 68
Controls (mean ±S D)	11 8±2 1	10 5±2 1*

* n=34 * n=25

substrate 4-methylumbelliferyl-α D-galactopyranoside according to Rietra et al (17) One unit of enzyme activity is defined as 1 μmol substrate hydrolyzed per hour at 37°C

The activities of α galactosidase and β-N acetylhexosaminidase were determined in fresh urine using the colorimetric substrates p nitrophenyl-α D-galactopyranoside and p nitrophenyl N acetyl β-D-glucosaminide according to Rietra et al (20) Each activity was measured with 0 15 ml and 0 25 ml enzyme preparation prepared from human urine as described previously (20)

In urine the ratio N acetyl β-glucosaminidase/α-galactosidase was calculated which is a reliable method of estimating the enzyme activity independent of urine concentration (20) α galactosidase activities in spleen liver and kidney were assayed according to Rietra et al (19) Artificial substrates were purchased from Koch Light Colnbrook England

Lipid analysis

Glycosphingolipids were determined in plasma according o the method of Vance and Sweeley (25) including silicic id chromatography alkaline methanolysis thin layer romatography acid methanolysis and gas liquid chromatography

For estimation of the lipid content in fresh-frozen human tissues the material was first subjected to aqueous homogenization freeze-drying and extraction with 8 volumes of chloroform/methanol (2 1) for 16 h at room temperature After filtration the residue obtained was extracted once more with 2 vol of chloroform/methanol (2 1) overnight and the pooled fractions partitioned according to Folch et al (9) The neutral glycosphingolipids present in the lower phase were analysed further according to Vance and Sweeley (25)

The glycosphingolipid content was measured by gas chromatographic determination of the trimethylsilyl derivatives of the methyl sugars by addition of pyridine bis-(trimethylsilyl)-trifluoroacetamide trimethylchlorosilane (10 10 2) Excess HCl was first removed by adding silver carbonate and sphingosine fatty acids and related compounds were removed by three successive extractions with n hexane Gas-liquid chromatography was carried out on a Carlo Erba D gas chromatograph on a column containing 3% SE 30 using a linear temperature program from 160 to 220 C with mannitol as an internal standard An example of the resolution is given in Fig 2 which shows the peaks obtained with the trimethylsilyl derivatives of mannitol and of the methyl glycosides from GL 3

In order to be sure of the reliability of the assay of glycosphingolipids in the plasma parallel determinations in plasma from healthy control individuals were always carried out the concentration of glycosphingolipids in control plasma being rather constant (24 25) The latter values were always within the expected range (see below) The concentration of each of the glycosylceramides was determined with a precision of about 15%

Pyridine was purchased from British Drug Houses trimethylchlorosilane (TMCS) and N O bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) from Pierce Chemical Company (Rockford USA) All other reagents used were of analytical grade

RESULTS

α galactosidase activity in plasma and serum

Table I shows the α galactosidase activity in serum or plasma of the patient before and after transplantation The activity 133 days before transplantation 0 33 mU/ml serum is 3% of that in control serum After transplantation the activity never exceeded 6–7% of the mean control value in serum and plasma

Neutral glycosphingolipids in plasma

In normal plasma the levels (means ±S D) of GL 1 Gl 2 GL 3 and GL-4 are 0 74±0 15 0.58 ±0 14 0 33 ±0 09 and 0 18±0 04 μmoles/100 ml plasma respectively (n=10) These values are comparable with those found by Vance et al (24 25)

The GL 3 level in the patient s plasma before transplantation was 2–6-fold higher than in normal plasma as expected (3 24) and 1 5–3 fold higher on the first and sixth days after transplantation than before (Fig 3)

On the 12th and 84th days after transplantation the GL-3 level declined markedly Subsequently the levels of GL 3 tended to increase once more as indicated by the two measurements at 174 and 229 days Forty days later the GL 3 level had declined again It is significant that the fluctuations in the level of GL-3 were paralleled by similar fluctuations in the levels of the other glycosphingolipids not only after transplantation but also in the preceding period

Neutral glycosphingolipids and α-galactosidase in urine

On thin layer chromatograms of lipid extracts pre pared from urinary sediment according to Desnick et al (8) no abnormal amounts of GL 3 or GL 2b could be detected In addition the α galactosidase activity in the urine 8 months after transplanta tion was normal the ratio β hexosaminidase/α galactosidase was 16 which is within the nor mal range of 1 2–20 5 (mean 7 4) (20)

Neutral glycosphingolipids in the tissues

Table II shows the glycosphingolipid concentra tions in various tissues obtained after autopsy and Table III the *n* fold increase in GL 1 GL 2 GL 3 and GL-4 compared with normal values Massive amounts of GL 3 could be demonstrated in spleen and liver (6–7 and 15 times the normal value respectively) GL 2b was found in pancreas and in the transplanted kidney but not in the other tissues tested Since no quantitative data on glycosphingo-lipid levels in pancreas are available the degree of accumulation of GL 2b and GL 3 in the patient is unknown

In aorta GL 3 was the only neutral glycosphingo-lipid present in a detectable amount 13 times that in the femoral artery of normal persons as reported by Philippart (14) Except for an increased concen tration of GL 1 (3 fold) the kidney revealed a normal glycosphingolipid pattern indicating that the normal allograft was able to break down any GL 3 presented to it Interestingly the enhance ment of the concentration of glycosphingol pids other than GL 3 found in plasma was also ob served in spleen and liver (Table III)

α galactosidase activity in various tissues

In order to investigate a possible effect of the kidney allograft in supplying normal α galacto

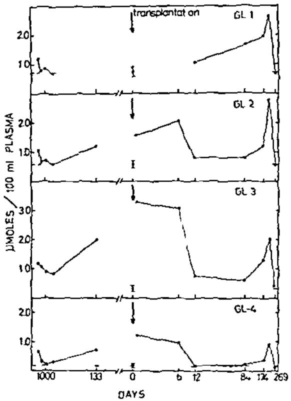


Fig 3 Plasma levels of GL 1 GL 2 GL 3 and GL-4 of the patient before and after renal transplantation – – – =mean normal values the bars=ranges

sidase to the lysosomal system of other tissues the α galactosidase activity was measured in autopsy material This material as well as control tissue was stored at −20°C for a few months It must be emphasized that during this time α galactosidase activity remains stable (19) As shown in Table IV the α galactosidase activities in spleen and liver were identical to those found in the tissues from an untreated Fabry hemizygote while the enzyme activity of the transplanted organ was normal as expected

Table II *Glycosphingolipid content (nmole/mg dry wt) in various tissues 280 days after renal transplanta tion*

	GL 1a	GL 1b	Gl 2a	GL 2b	GL 3	GL-4
Liver	1 08	0 31	0 67	N D	3 06	0 51
Spleen	1 25	<0 25	1 71	<0 04	1 76	0 71
Pancreas	0 64	1 23	0 73	1 39	1 70	–
Aorta	N D	N D	N D	N D	1 00	N D
Kidney	0 79	0 54	0 38	0 46	0 80	0 80

N D =not detectable

DISCUSSION

In Fabry s disease trihexosylceramide accumulates in the various tissues and body fluids to a different extent There is a six fold increase in plasma (3 24) and a sixty fold increase in kidney (13 21) Large amounts have also been reported in the arterial walls (13 14) Although deficiency of ceramide trihexosidase is ubiquitous it is clear that the extent and rate of accumulation of sphingolipid depend on several factors such as the actual synthesis in a given cell the rate of turnover the uptake of exogenous lipid the possibility of excretion of lipid material and the life span of the cell (14) In this respect the increased plasma lipid content might be of special importance The enhanced concentration in plasma could function as an exogenous factor contributing to the lipid accumulation in other tissues

Little is known however about the source transport and clearance of sphingolipids from the circulation From the studies of Dawson and Sweeley (5) carried out on porcine blood it is likely that erythrocytes are the main source of glycosphingolipids in the plasma

Table III *n fold increase in glycosphingolipid concentrations in various tissues 280 days after renal transplantation compared with normal tissues*

Normal values in liver and kidney according to Kint (11) in aorta according to Philippart (14) and in plasma and spleen from our own measurements

	GL 1	GL 2	GL 3	GL-4
asma	2–3	2	5–6	3–4
pleen	3–4	3	6–7	2
Kidney	3	1 5	1	1
Liver	3–4	2	15	3–4
Aorta	N D	N D	13	N D

N D =not detectable

Table IV *α-galactosidase activity (U/g wet wt) in normal and Fabry tissues*

	Normal tissue	Tissue of *untreated* Fabry hemizygote	Tissue of the present patient*
Liver	4 68	0 61	0 65
Spleen	4 42	0 21	0 25
Kidney	4 86	0 42	4 67

* 269 days after transplantation.

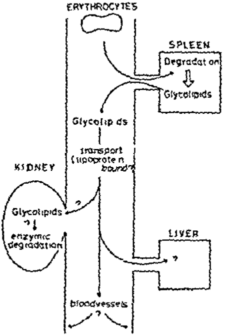


Fig 4 Possible pathways for the turnover of neutral glycosphingolipids in the circulation

Evidence exists that the plasma glycosphingolipids are localized in the low and high-density lipoprotein fractions (Van den Bergh in preparation 22) It is important in this respect to mention the results of Bierman and Albers (2) who showed that human arterial smooth muscle cells can carry out endocytosis of low density and very low density lipoproteins this might explain the relatively vast amounts of GL 3 present in the arterial walls of Fabry patients This pathway for the accumulation of GL 3 is shown schematically in Fig 4

Although the indication for renal transplantation in Fabry s disease is primarily uremia *it has been* suggested that a concomitant lowering of the lipid level in the circulation might have positive effects in slowing down the accumulation process The rationale is that by implanting a healthy renal allograft a continuous source of enzyme is introduced capable of degrading accumulated material either in the renal tissue itself or in other organs supplied with normal enzyme derived from the kidney

Kidney transplantations in Fabry s disease accompanied by measurement of enzyme activity and lipid concentrations have been carried out by Philippart et al (14 15 16) Desnick et al (6 7) Clarke et al (4) and Wolfe et al (26) However

their results are not unequivocal (18) Both transplantations carried out by Desnick et al resulted in a decline in plasma GL 3 to normal values within 1–2 months after transplantation The α galactosidase activity in the plasma rose from approximately 2% to 10% of the normal value In one patient the renal allograft was examined post mortem and exhibited normal levels of all glycosphingolipids Furthermore α galactosidase activity in urine was normal and there were no lipid abnormalities In both patients the bouts of pain typical of Fabry s disease were reported to have disappeared and the ability to perspire seemed to have increased (6)

The results of one of the transplantations carried out by Philippart et al (14 15 16) agree with these data with respect to the enzymic measurements in plasma and urine and the clinical improvements However although the GL 3 content of the plasma first decreased it subsequently rose to ab normal values During the last 6 months of the follow up period of 2½ years the plasma GL 3 content had declined again In the second patient treated by Philippart et al no effect on the plasma GL 3 content was noted during the follow up of 15 months apart from some marked fluctuations immediately following transplantation

By measuring both GL 3 and GL-4 values in the plasma of their transplanted patient Clarke et al (4) made the important observation that both glycosphingolipids increased and decreased in a parallel way They concluded that the kidney transplantation did not affect the catabolism of GL 3 but the supply *of its precursor* GL-4 An identical picture was found in our patient Moreover the striking parallelism in the decrease and increase was observed not only for GL 3 and GL-4 but also for the other neutral glycosphingolipids both before and after transplantation (Fig 3) The fluctuations in glycosphingolipid levels before transplantation may be related to the clinical picture of uremia for which the patient was treated by hemodialysis As shown in Table III a high level of GL 3 was found after autopsy in spleen and liver However there was also an increase in the other glycosphingolipids compared with the normal values We have no satisfactory explanation for this enhancement the parallelism between spleen plasma and liver suggests that a mutual relationship may exist caused by the release of glycosphingolipids from spleen in the circulation followed possibly by clearance from it by the reticuloendothelial system particularly in the liver (Fig 4)

Although our results support in part the suggestion of Clarke et al (4) that the supply of GL-4 is the main factor responsible for the fluctuations in the GL 3 concentration in plasma the concomitant fluctuations in GL 1 and GL 2 are more difficult to explain Renal transplantation introduces a number of factors that may affect glycosphingolipid turnover such as decreased uremia renewed erythropoietin synthesis administration of immunosuppressives and turnover of leucocytes (1)

We found no evidence for an increase in plasma α galactosidase activity following the transplantation or increase in α galactosidase activity in spleen and liver

Considering all the possible aspecific effects on glycosphingolipid metabolism that can occur in transplanted patients we feel that there is little if any evidence for a specific enzymic break down of accumulated GL 3 in Fabry patients following renal transplantation

With regard to the normalizing of the enzymic activity and GL 3 content of the urine it is clear that this is a reflection of the presence of a healthy, implanted kidney

Our results and those reported in the literature lead us to conclude that although uremia may be an indication for renal transplantation in patients with Fabry s disease it is very doubtful if the transplantation is effective in slowing down glycosphingolipid accumulation in the tissues of the patient

ACKNOWLEDGEMENTS

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Hypertension in End-stage Renal Disease

The Relationship between Blood Pressure Plasma Renin Plasma Renin Substrate and Exchangeable Sodium in Chronic Hemodialysis Patients

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ABSTRACT Blood pressure (BP) plasma renin concentration (PRC), plasma renin substrate concentration (PRSC) and exchangeable sodium (ES) have been studied in 27 patients undergoing regular hemodialysis because of end stage renal disease PRC was significantly higher in the hypertensive than in the normotensive patients The pattern of PRSC was similar in the groups of patients but with a marked individual variation ES was slightly lower in the hypertensives than in the normotensives but the difference was not statistically significant Multiple regression analysis demonstrated a significant correlation between mean BP, the natural logarithm of PRC and ES, but the effect of ES was negligible PRC was negatively correlated to ES in all patients, including the hypertensives These results strongly suggest that the renin–angiotensin system is the most important factor involved in the pathogenesis of hypertension in end stage renal disease when sodium balance is adequately controlled A clinical application of the predictive value of PRC concerning the effect of bilateral nephrectomy on hypertension is outlined

In the majority of patients with far advanced renal insufficiency and hypertension exchangeable sodium (ES) is increased (4 7 9) and blood pressure (BP) becomes normal when excess ES is removed by dialysis (1) The renin content of the plasma in these patients is normal or only slightly increased (2 3 20 22 23) In some patients BP cannot be adequately controlled by dialysis The renin content in the plasma in these patients is usually greatly increased and BP first becomes normal after bilateral nephrectomy (2 3 20–24) Thus a distinction has been made between so-called salt and water-dependent and renin-dependent hypertension in patients treated with chronic hemodialysis (22)

Results of previous experiments are however difficult to interpret partly because of differences in analytical methods used for measuring the plasma renin content Thus several studies have used plasma renin activity instead of plasma renin concentration (PRC) for this purpose In addition differences in the sodium status of the patients studied make it difficult to interpret results of previous experiments

In an effort to reduce the variables affecting the renin–angiotensin system in the present study PRC was measured using an internal standard human renin as reference and the patients studied were in optimal sodium balance The purpose of the study was to elucidate some of the pathogenetic and clinical aspects of the renin–angiotensin system and sodium balance in hypertensive end stage renal disease by studying the relationship between BP PRC plasma renin substrate concentration (PRSC) and ES in patients undergoing chronic hemodialysis

MATERIAL AND METHODS

Patients

Twenty seven patients were studied 15 women and 12 men aged 10–63 years mean 40 1±16 2 (1 S D) who at the time of study had been on regular intermittent hemodialysis treatment for 1–36 months mean 5 4±8 0 (1 S D) Ten patients had chronic glomerulonephritis 12 chronic pyelonephritis and 5 malignant nephrosclerosis polyarteritis nodosa polycystic renal disease and bilateral renal cortical necrosis All had 24-hour creatinine clearance <5 ml/min Daily urine volume averaged 349 ml/24 hours ±328 (1 S D) Mean sodium excretion in the urine was 13 8 mEq/24 hours ±14 7 (1 S D) At the time of study bilateral nephrectomy had not been performed in any case

The patients were divided into three groups Group A Four patients who were normotensive before and during

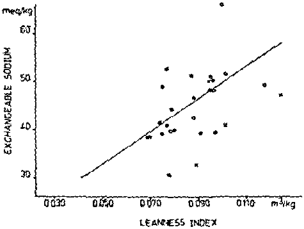


Fig 1 Relationship between leanness index (height²/weight) and exchangeable sodium in normals (●) compared with normotensive (O) and hypertensive (×) chronic hemodialysis patients The regression line for normals $y=341.9x+15.8$ $r=0.89$ $p<0.001$

hemodialysis treatment Their BP averaged 124/78 mmHg±12 5/12 6 (1 S D) and no hypertensive lesions were apparent Group B Thirteen patients who had been hypertensive before dialysis but who during dialysis became normotensive without antihypertensive therapy Their BP averaged 136/82 mmHg±16 2/8 5 (1 S D) Two patients had grade III hypertensive retinal changes 3 had hypertrophy and/or strain on ECG and in 8 the cardiac silhouette was enlarged on X ray Group C Ten patients who in spite of optimal dialysis treatment had persistent hypertension i e diastolic BP≥100 mmHg BP was 179/114 mmHg±20 9/11 8 (mean±1 S D) Three patients had grade III hypertensive retinal changes 7 hypertrophy and/or strain on ECG and 7 cardiac enlargement on X ray

Glomerulovascular and tubulointerstitial renal diseases were present with equal frequency in the hypertensive (group C) and normotensive patients (groups A and B) ($p>0.2$ Fisher's exact test)

Procedure

Regular intermittent hemodialysis involved 2 weekly dialyses of 8–10 hours An attempt was made to hold the patients at dry weight i e the weight where edema was not present and where further weight reduction would lead to hypotension (22)

All patients were studied during their hospital stay Antihypertensive treatment and/or diuretics were discontinued at least 3 weeks before study which was performed between dialyses over a period of at least 3 days During this period they received a diet low on protein and salt with a fixed sodium content of 10 mEq/24 hours BP was measured at 8–9 a m before the start of dialysis at the end of the period of study Simultaneously blood specimens were taken for determination of PRC and PRSC after bed rest and fast for 8 hours ES was measured on the day before dialysis at the end of the study period

The patients were informed about the purpose and character of the study and all were willing to participate

Methods

Plasma renin concentration was measured using the method described by Giese et al (10) This method involves radioimmunoassay of angiotensin I after incubation of dialysed plasma at 37°C and Ph 7 4 both with and without a standard human renin and extraction of the angiotensin I produced PRC is expressed in micro Goldblatt Units per ml plasma (μGU/ml) using the above mentioned standard human renin as reference In 15 normal persons (11 men and 4 women) with a mean age of 27 8 years (range 17–58) mean PRC was 36 5 μGU/ml±16 4 (1 S D) (range 10–79) during basal conditions i e at 8–9 a m after 8 hours fasting and 1 hour's rest in the supine position but otherwise on a liberal salt diet In 6 anephric patients mean PRC was 6 0 μGU/ml±5 8 (1 S D) (range 0–16) The coefficient of variation for the reproducibility of the analysis (day to day) was 11% within the range of 10–175 μGU/ml

Plasma renin substrate concentration was measured using the method described by Giese et al (10) The technique involves radioimmunologic measurement of the angiotensin I produced during incubation of dialysed plasma after addition of highly purified human renin in an amount sufficient for complete consumption of the renin substrate present The amount of renin substrate is expressed as ng angiotensin I per ml plasma (ng ang I/ml) In 14 normal persons (11 men and 3 women) with a mean age of 31 4 years (range 23–65) mean PRSC was 1408 ng ang I/ml±203 (1 S D) (range 1 178–1 796)

Dr J Giese et al Dept of Clinical Physiology KAS Glostrup Hospital Copenhagen Denmark placed antiserum against angiotensin I and highly purified human renin at our disposal and the Medical Research Council Division of Biological Standards National Institute for Medical Research Mill Hill London provided Renin Human 68/356

Exchangeable sodium was measured using an isotope dilution technique as described by Hansen (11) ^{24}Na (approximately 100 μCi) was given i v after removal of a standard Activity in the standard serum and urine was measured after 24 hours of equilibration ES was calculated according to the formula

$$\frac{\text{administrated activity} - \text{activity in the urine}}{\text{activity in the plasma/ml} \times 1000} \times \text{serum}$$

sodium concentration (mEq/ml)

The serum sodium concentration was measured by flame photometry The regression line (given in Fig 1) for the relationship between ES (mEq/kg) and leanness index (height²/weight) in 16 normal persons (8 men and 8 women mean age 49 2 years range 20–65) was used as reference ES values measured in this study were expressed as a percentage of expected ES in normals with the same "leanness index" (8)

Statistics Mann Whitney's rank sum test was used for comparison between groups and Separman's test for estimating the correlation between paired observations Regression analysis was performed according to the method described by Nie et al (16) Danish Agricultural EDP-Center Århus Denmark performed the regression analysis

Table I *Mean values of blood pressure (BP) plasma renin concentration (PRC) plasma renin substrate concentration (PRSC) exchangeable sodium (ES) height body weight and leanness index in normotensive (groups A and B) and hypertensive (group C) chronic hemodialysis patients*

	BP (mmHg)	PRC (μGU/ml)	PRSC (ng ang I/ml)	ES (%)	Height (cm)	Weight (kg)	Leanness index (m^3/kg)
Group A							
Mean	124/78	31 3	2 206	101 6	171 0	56 2	0 090
±S D	13/13	5 3	211	18 1	4 8	3 1	0 009
±S E M	6/6	2 6	122	9 0	2 4	1 6	0 005
Group B							
Mean	136/82	73 7	1 764	99 4	167 2	53 5	0 091
±S D	16/9	76 4	600	15 7	13 9	14 8	0 012
±S E M	5/2	21 2	166	4 3	3 8	4 1	0 003
Group C							
Mean	179/114	439 0	1 944	89 7	165 9	55 2	0 087
±S D	21/12	424 1	951	12 0	10 9	13 0	0 018
±S E M	7/4	134 1	301	3 8	3 4	4 1	0 006

RESULTS

Results are summarized in Table I

Plasma renin concentration was significantly higher in hypertensive patients in group C than in normotensive patients in groups A and B ($p<0$ 01) PRC was not significantly different in patients in groups A and B The variation in PRC in groups B and C was great with some overlapping

Plasma renin substrate concentration The pattern of PRSC was similar in the three groups of patients with marked individual variation

Exchangeable sodium averaged 10 2% less in patients in group C than in patients in groups A and B but the difference was not statistically significant (0 10>p>0 05) The individual values of ES are shown in Fig 1 which demonstrates that compared with normals the variation of ES was great

The relationship between BP PRC and ES Mean BP (diastolic BP+1/3 of the BP amplitude) was positively correlated to PRC (rho=0 58 $p<0$ 01) No correlation was found between mean BP and ES Multiple linear regression analysis using mean BP as the dependent variable and the natural logarithm of PRC (x_1) and ES (x_2) as the two independent variables demonstrated a significant correlation ($y=11$ $56x_1+0$ $24x_2+36$ 91 $r=0$ 64 $p<0$ 001) A negative correlation was found between PRC and ES in all patients (rho=−0 42 $p<0$ 05) but not in patients in each group when considered separately

DISCUSSION

The results of this study show that abnormal salt and water balance and abnormal activity of the renin–angiotensin system are important pathogenetic factors for the hypertension associated with end stage renal disease BP became normal on optimal dialysis treatment in patients with normal or slightly increased PRC (group B) whereas hypertension persisted in patients with markedly increased PRC (group C) in spite of optimal dialysis A positive correlation was found between BP and PRC in all patients studied

These findings are in agreement with those of previous studies (2 3 19–24) and support a distinction between so-called salt and water-dependent and renin-dependent hypertension (22) Whereas some investigators have found a complete separation between plasma renin values in the two forms of hypertension (3 22 23) others including the present author have found a certain amount of overlapping (2 19 20 24) while some have not been able to demonstrate any difference (6 12 17) Wilkinson et al (24) Chrysanthakopoulos et al (3) and Verniory et al (21) found a positive correlation between BP and plasma renin as in the present study whereas Kotchen et al (12) and Crasswell et al (6) could not demonstrate such a correlation This discrepancy between previous studies may be due to differences in the analytical techniques used

to measure plasma renin content and to variations in the sodium status of the patients studied

The method used to measure plasma renin in the present study was independent of the plasma renin substrate content and other factors which inhibit or activate the renin–angiotensin system This is in contrast to most of the previous studies where plasma renin activity was measured PRC in this study was therefore an expression of plasma renin content alone and the great variation in renin substrate content in the plasma demonstrated in this study in patients with end stage renal disease can be safely excluded as contributing to changes in plasma renin content In addition the present results may be easily compared with those of others if the standard human renin used here is generally employed

Owing to loss of the kidneys ability to excrete salt and water the salt and water balance was regulated primarily by dialysis and dietary restrictions in the patients in this study Almost all patients were in sodium balance since the oral sodium intake approximated the sodium excretion in the urine and clinically all patients were held at optimal weight and no significant difference was found between ES in the groups of patients This suggests that variations in sodium and water balance between the groups were negligible and alterations in the renin–angiotensin system caused by such variations can for all practical purposes be ruled out On the other hand variations in ES were rather large compared with normals showing how difficult it is to judge sodium status in these patients on the basis of a clinical evaluation alone

The basis used for reference is of great importance when evaluating ES values since ES is lower in overweight individuals because of the low sodium content of adipose tissue A leanness index as employed here is recommended by others for use in patients with renal insufficiency because of their frequently abnormal salt and water balance (8) The relationship between ES and leanness index in normals in this study was almost identical to that found by Nicholson and Zilva (15) and Davies et al (8)

Davies et al (8) and Rosen and Robinson (18) found an inverse relationship between plasma renin and ES in normotensive but not in hypertensive patients with renal insufficiency and malignant hypertension and a better separation of normotensive and hypertensive patients when plasma renin was expressed in relation to ES indicating that plasma renin is inappropriately high in patients with renal hypertension In contrast to these studies a negative correlation between PRC and ES was found in all our patients including the hypertensives and PRC expressed in relation to ES did not contribute to an additional separation of the patient groups Thus the present study does not support the concept of inappropriately high plasma renin

In this study multiple regression analysis between BP PRC and ES indicates that plasma renin is the most important factor involved in the pathogenesis of hypertension in chronic hemodialysis patients whereas the significance of ES is negligible when sodium balance is under adequate control However the inverse correlation found between PRC and ES suggests that salt balance may modify renin release

The reason for the increased release of renin in some patients with end stage renal disease is unknown In a review of the literature Weidmann et al (23) found that 86% of dialysis patients with high plasma renin and so called uncontrollable hypertension had glomerulovascular renal disease which suggests that increased renin release may be related to the nature of the renal disease This was not supported by the present findings Brown et al (2) mention that increased renin release is possibly initiated and accelerated by extreme salt and water depletion as a consequence of very intensive dialysis and leads by way of a self increasing mechanism to uncontrollable hypertension Such a mechanism is supported by the observation that ES tended to be lower in the hypertensive than in the normotensive patients and by the inverse correlation found between PRC and ES in all the patients including the hypertensives

Hypertension which cannot be controlled by elimination of salt and water and which is often accompanied by a marked increase in plasma renin has been referred to as uncontrollable in previous studies and bilateral nephrectomy found advisable (3 13 14 20 22 24) In contrast most of the patients in the present study with high PRC had moderate hypertension which was easily controlled with conventional antihypertensive treatment In only two patients with the highest PRC values was hypertension so severe that it could be characterized as uncontrollable and bilateral nephrectomy was carried out Thus in agreement with

Crasswell et al (5 6) it appears that severe uncontrollable hypertension is rare in patients undergoing chronic hemodialysis On the basis of the present study it is concluded that bilateral nephrectomy is only indicated in the few patients with excessively high PRC and persisting hypertension despite adequate dialysis and where oral antihypertensive therapy is ineffective In the majority of patients with high PRC and in all patients with normal PRC hypertension is controllable and bilateral nephrectomy unnecessary

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Table 1 *Plasma aldosterone concentration (PAC) plasma potassium plasma sodium BP and plasma renin concentration (PRC) before (A) and during treatment with alprenolol (B)*

Pat no	PAC (ng/100 ml)		Plasma potassium (mEq/l)		Plasma sodium (mEq/l)		BP (mmHg)		PRC (µGU/ml)	
	A	B	A	B	A	B	A	B	A	B
1	15 5	11 9	3 6	4 0	140	140	180/125	150/110	49	15
2	27 9	19 7	3 2	3 5	141	143	170/120	160/110	28	5
3	17 8	14 8	4 3	4 5	132	142	190/120	170/110	24	30
4	20 2	16 5	3 5	3 8	137	140	210/130	180/120	36	8
5	13 9	16 4	4 6	3 9	143	141	190/125	180/130	31	14
6	14 0	12 9	4 0	3 9	141	142	200/110	190/110	40	30
7	21 8	17 2	4 0	4 0	140	139	160/125	130/100	24	35
8	15 8	16 4	4 1	3 6	138	140	160/110	130/90	39	34
9	23 9	20 9	4 4	5 2	140	145	160/110	135/95	23	9
10	27 7	18 6	3 4	3 6	142	140	160/115	140/100	116	50
11	17 1	8 8	3 8	4 3	139	134	200/130	200/130	45	12
12	25 9	17 6	4 1	4 7	141	144	170/110	170/110	28	21
13	18 2	12 8	3 9	3 7	137	143	220/120	170/120	26	23
14	8 7	3 7	3 0	3 2	145	145	170/115	180/110	7	5
15	31 8	21 2	3 2	4 1	140	142	180/115	145/95	60	26
16	16 0	13 5	4 1	4 4	136	137	210/125	190/115	21	28
17	8 1	4 5	3 6	3 6	141	138	180/120	170/115	14	40
18	25 2	29 0	3 8	3 9	140	141	160/110	140/95	35	46
19	14 7	10 8	3 8	4 1	137	137	150/110	135/95	25	24
20	13 9	3 8	4 4	5 8	140	140	210/130	190/120	29	15
21	34 8	17 4	4 0	4 6	139	140	200/130	190/115	28	29
22	15 9	19 0	3 7	4 1	141	141	170/125	135/110	55	28
Mean	19 5	14 9	3 8	4 1	140	141	182/120	163/109	36	24
S D	7 1	6 1	0 4	0 6	2 7	2 7	20/8	23/11	22	13

Procedure

All participants were out patients on liberal sodium and potassium intake and all were seen weekly for 2 months *The first month was a control period during which all* drugs were discontinued During the second month the patients were treated with alprenolol (Aptin Duretter®) verage dosage 964 mg/day divided into two or three ly doses

Blood samples for determination of PAC PRC sodium nd potassium were collected at the end of the control period and after one month s treatment with alprenolol Blood was drawn in the morning at 9 a m after the patients had been in the supine position for at least one hour and after approximately 8 hours fast Urine was collected for a 24-hour period on the day before blood sampling

METHODS

PAC was measured using Damkjær Nielsen s method (13) —a radioimmunological measurement performed on plasma after previous extraction with dichloromethane purification on silica gel columns and chromatographic separation on paper The position of aldosterone on the paper was located by scanning Aldosterone antiserum was obtained from Research Plus Laboratories Denville New Jersey The coefficient of variation was 15% PAC in 23 normal healthy control subjects was 13 5 ng/100 ml (range 4 6–25 9) after one hour in the supine position PAC in samples from the same patients was determined during the same analytic run

PRC was measured using the method described by Giese et al (9) This involves radioimmunological measurement of angiotensin I after previous dialysis of plasma incubation at 37°C and pH 7 4 both with and without addition of a human standard and extraction of angiotensin I produced PRC is given in Goldblatt units (GU) using the above mentioned human renin as reference Dr J Giese Department of Clinical Physiology Amtssygehuset Glostrup Denmark provided antiserum against angiotensin I and the Medical Research Council Division of Biological Standards National Institute fo Medical Research Mill Hill London placed Renin Human 68/356 at our disposal The coefficient of variation was 11% PRC in 15 healthy control subjects was 3 µGU/ml (range 10–79)

Sodium potassium and creatinine in serum and urine were determined by conventional AutoAnalyzer technique (Dept of Clinical Chemistry Kommunehospitale Århus Denmark)

BP was measured by sphygmomanometer after at lea one hour s rest in the supine position Several measur ments were performed at each session and the lowe readings were used Mean BP was calculated as diasto pressure plus ⅓ of the pulse pressure

Wilcoxon s signed rank test and Spearmann s test w used in the statistical calculations

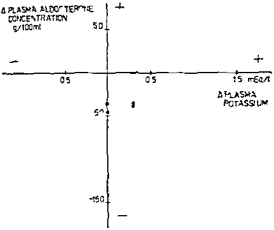


Fig 1 Relationship between changes in plasma aldosterone concentration and changes in plasma potassium induced by alprenolol in 22 patients with essential hypertension

RESULTS

Table I shows PAC PRC plasma potassium plasma sodium and BP before and after treatment with alprenolol PAC decreased significantly from 19 5±7 1 (1 S D) to 14 9 ng/100 ml±6 1 (1 S D) ($p<0\ 01$) PRC decreased significantly from 36± 22 (1 S D) to 24 μGU/ml±13 (1 S D) ($p<0\ 02$) Plasma potassium increased significantly from 3 8±0 4 (1 S D) to 4 1 mEq/l±0 6 (1 S D) ($p<0\ 01$) Both systolic and diastolic BP were significantly reduced during treatment with alprenolol from 182/120±20/8 (1 S D) to 163/109 mmHg± 23/11 (1 S D) ($p<0\ 01$)

No significant changes were measured in plasma sodium concentration 140 before and 141 mEq/l after treatment ($p>0\ 05$) The 24 hour urinary excretion of potassium and sodium were very similar before and after treatment for potassium 64±42 (1 S D) before and 55 mEq/l±22 (1 S D) after ($p>0\ 05$) and for sodium 115±59 (1 S D) before and 117 mEq/l±73 (1 S D) after ($p>0\ 05$)

Plasma potassium and PAC were not significantly correlated either before (*rho*=−0 147 $p>0\ 05$) or after treatment with alprenolol (*rho*=0 088 $p>0\ 05$) Fig 1 shows however that changes in PAC were significantly correlated to changes in plasma potassium concentration (*rho*=−0 516 $p<0\ 02$)

Plasma sodium and PAC were not significantly correlated either before (*rho*=−0 138 $p>0\ 05$) or after treatment (*rho*=0 201 $p>0\ 05$) Changes in plasma sodium did not correlate with changes in PAC (*rho*=−0 040 $p>0\ 05$)

PAC was not correlated to mean BP (*rho*=−0 139 $p>0\ 05$) before treatment During alprenolol treatment however a significant correlation was found between mean BP and PAC (*rho*=−0 471 $p<0\ 05$) (Fig 2) Changes in mean BP were not correlated to changes in PAC (*rho*=−0 118 $p>0\ 05$)

PRC was not correlated to mean BP either before (*rho*=0 079 $p>0\ 05$) or after alprenolol treatment (*rho*=−0 347 $p>0\ 05$) and changes in PRC were not correlated to changes in mean BP (*rho*=0 159 $p>0\ 05$)

DISCUSSION

The present results show that one month s therapy with alprenolol induces a significant reduction in BP associated with a decrease in both PAC and PRC and an increase in plasma potassium whereas plasma sodium remains unchanged Evidence of decreasing secretion of aldosterone during treatment of hypertensive patients with a β-adrenergic blocking agent has also been obtained by Buhler et al (3) who found a decreasing urinary excretion of aldosterone in patients with essential hypertension during treatment with propranolol They suggested that the decrease in PRA and urinary aldosterone excretion produced by propranolol bore a causal relationship to the antipressor effect Although this is possible it should be mentioned that neither the present study nor several others (2 10 12) have been able to demonstrate a significant correlation between the suppression of plasma

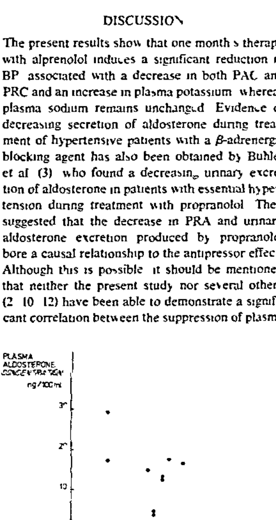


Fig 2 Relationship between mean BP and plasma aldosterone concentration in 22 patients with essential hypertension treated with alprenolol

renin and the BP reduction during β adrenergic blockade In this study statistical analysis did not reveal any correlation between levels of PAC and BP during the control period During alprenolol treatment however a significantly inverse correlation was found between these parameters Thus the lower the BP obtained the higher the PAC In all but one patient (no 18) however PAC was within the normal range after treatment The results suggest that PAC is not of primary importance for the regulation of BP during alprenolol treatment

Although plasma sodium and ACTH influence aldosterone secretion the renin–angiotensin system and plasma potassium are the most important regulatory factors of aldosterone secretion (15 17) Plasma potassium appears to be the dominant factor influencing aldosterone secretion in patients with primary hyperaldosteronism (14) and in anephric man (1) conditions where plasma renin is very low According to Buhler et al (3) the renin suppressive action of β adrenergic blocking agents constitutes a pharmacological analogue to nephrectomy

With regard to the above it might be supposed that plasma potassium was the most important regulatory factor for the aldosterone secretion in patients treated with β adrenergic blocking agents In the present study we found an increase in plasma potassium during alprenolol treatment but no correlation was found between PAC and plasma potassium concentration either before or after this treatment It has been shown that potassium has direct effect on aldosterone secretion (8) and thus rise in the level of plasma potassium might stimulate and a fall inhibit aldosterone production An increase in plasma potassium during alprenolol treatment might be accompanied by an increase in PAC However the present results have shown that the opposite takes place i e a decrease in PAC in spite of an increase in plasma potassium The changes in PAC induced by alprenolol treatment correlate inversely with the changes in plasma potassium and the results suggest that plasma potassium may have some importance for the regulation of aldosterone secretion during alprenolol treatment Since however the correlation between changes in plasma potassium and PAC is negative plasma potassium cannot be the sole regulatory factor and the changes in PAC during β adrenergic blockade must be attributed to other aldosterone regulatory factors

Most studies show that plasma sodium is of little importance for the regulation of aldosterone secretion This agrees with the present results in which no relationship could be demonstrated between plasma sodium and PAC either before or during alprenolol treatment

Changes in sodium and potassium intake induce changes in PAC (5 11) All patients in this study were on a liberal sodium and potassium intake but the 24 hour urinary excretion of sodium and potassium was unaffected by alprenolol therapy All patients were examined after at least one hour in the supine position at the same time in the morning Differences in posture as a cause of the changes in PAC can thus be ignored

It has been shown earlier that ACTH can stimulate aldosterone secretion (7 16) The dose required however induces extemely high levels of ACTH compared with the level under normal physiological circumstances Since all patients were investigated at the same time in the morning the hypothetical influence of changes in PAC due to the circadian rhythm in ACTH secretion can be ruled out

In good agreement with previous reports (2 3 6 10 12) the plasma content of renin was suppressed by β adrenergic blockade This suppression however was not always to very low values either in this study or in the studies mentioned above Thus the renin–angiotensin system may still influence the aldosterone secretion during β adrenergic blockade The relationship between PAC and plasma potassium during β adrenergic blockade appears to be interfered by the renin–angiotensin system and β adrenergic blockade may not represent a pharmacological analogue to bilateral nephrectomy

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The Effects of Different Dose Regimens of Nicentrol on Serum Lipid Concentrations in Man

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ABSTRACT The lipid lowering effects of 3 g of the nicotinic acid derivative pentaerythritoltetranicotinate (nicentrol) given either 1 g×3 or 1 5 g×2 have been evaluated in 18 subjects with hyperlipoproteinaemia When 1 g nicentrol was given three times daily, the serum TG concentration fell from 3 14±0 48 to 1 86±0 18 mmol/l (41% reduction) and the serum cholesterol concentration from 282±9 to 227±11 mg/100 ml (20% reduction) The same daily dose, given 1 5 g twice did not significantly lower the serum TG concentration and serum cholesterol was lowered by only 12% Nicentrol tablets prepared with a dissolution time of 60 or 90 min had identical lipid lowering properties Although patients may find it practical to take nicentrol only twice daily such a dose regimen has considerably less effect on elevated serum lipids than a thrice daily regimen

Nicotinic acid and some of its derivatives have been used extensively in the treatment of hyperlipoproteinaemia and have been found to be effective in lowering elevated concentrations of both serum triglycerides (TG) and cholesterol (2 6 7) In a series of studies we have investigated the lipid lowering properties of the derivative pentaerythritoltetranicotinate (nicentrol) which has clinical effects almost identical to nicotinic acid but less side effects (6 7) A great clinical advantage with nicotinic acid and nicentrol treatment is the dose–response effect which usually makes it possible to increase the dose until satisfactory serum lipid levels are obtained (7)

Since many patients on nicentrol treatment are employed people and are often treated basically with diets before nicentrol is added they may have difficulties in taking the drug in the middle of the day because of unpleasant side effects such as cutaneous flush and gastrointestinal upsets In order to avoid those complications we have studied the feasibility of administering nicentrol twice instead of thrice daily which is the generally recommended dosage

Combined treatment with clofibrate and nicentrol has a synergistic lipid lowering effect (7) Clofibrate used in such combinations is generally given twice daily and it would therefore be practical if the combining nicentrol could be given with the same scheme After absorption in the upper part of the gastrointestinal tract nicentrol is hydrolyzed to nicotinic acid Nicentrol tablets (Perycit®) are manufactured so that at least 90% of the drug is released within 60 min in gastric juice Side effects of nicotinic acid and its derivatives have been attributed to a fast increase in the plasma concentration of nicotinic acid For this reason we also investigated whether tablets with a slower dissolution rate would cause less gastrointestinal side-effects but have the same effect on serum lipids

MATERIAL AND METHODS

Eighteen patients who had all been previously treated with nicotinic acid were studied at the Lipid Unit Karolinska Hospital No lipid lowering drugs were given for at least two months before entry into the study Fasting serum lipids were determined twice with one month s interval and Perycit® 3 g/day was then administered either as 2 tablets thrice or as 3 tablets twice daily Treatment was started with either dose regimen at random order After one month the patients changed dose regimen Serum

Table I *Effects of nicentrol 3 g/day divided in either two or three doses and prepared with different dissolution times on serum TG and cholesterol concentrations (mean ±S E M pretreatment values are means of two determinations)*

	TG (mmol/l)	Cholesterol (mg/100 ml)
Perycit® in 2 or 3 daily doses (n=18)		
Before treatment	3 14±0 48	282±9
1 g thrice	*1 86±0 18*	*227±11*
1 5 g twice	2 51±0 40	248±10
Nicentrol prepared with different dissolution times (n=8)		
Before treatment	2 95±0 30	273±15
60 dissolution time	1 91±0 31	236±16
90 dissolution time	2 08±0 22	228±6

lipids were determined at the end of each month After treatment with Perycit 8 patients continued the programme with nicentrol prepared with 90 min dissolution time for one month Serum TG and cholesterol were determined with AutoAnalyzer methods (1 4)

RESULTS

Since the patients had been selected on the grounds that they tolerated nicentrol all could complete the study without serious side effects However most patients preferred the twice-daily regimen which was practical to take caused less gastrointestinal *problems and also reduced the risk of late flushes* after drug administration No systematic preferences were expressed concerning nicentrol tablets with various dissolution times

Serum lipids before and during treatment are summarized in Table I When entering the study most patients had increased serum TG concentrations in some cases with a concomitant increase in serum cholesterol Only two subjects had an isolated increase in the serum cholesterol concentration

Perycit in the recommended dose significantly *reduced serum TG by 41% ($p<0$ 05) and serum* cholesterol by 20% ($p<0$ 001) However when the dose was given twice daily no significant reduction *in the serum TG level was seen The serum choles*terol reduction on a twice-daily dosage was still significant ($p<0$ 001) but only 12% When twice daily and thrice-daily values were compared statistically by the method of Student s test for paired differences serum TG as well as cholesterol levels were significantly higher during the twice-daily regimen ($p<0$ 05 $p<0$ 01 respectively) The dissolution time of the nicentrol tablets did not significantly affect serum lipid reduction (Table I)

DISCUSSION

A major problem during treatment with nicotinic acid is the difficulty of overcoming the side-effects Although flush generally is a smaller problem after long term treatment with nicentrol (6) the gastrointestinal side effects often do not disappear with time In the present study on patients who were known to tolerate nicentrol the lipid lowering effects of 1 g thrice daily was in agreement with our earlier findings (6 7) However when the same *dose was given twice daily this effect on serum TG* was completely abolished and the effect was less on serum cholesterol The underlying mechanisms for this diminished effect remain to be clarified One possible explanation can be derived from the studies of Carlson et al (3) who postulated that the *reduction in FFA found after nicotinic acid ad*ministration leads to a reduction in hepatic TG synthesis and secretion of the TG rich very low density lipoproteins (VLDL) from the liver This VLDL decrease leads to a reduction in its product the low density lipoproteins which are rich in cholesterol (5) Nicentrol twice daily will lead to longer periods of low concentrations of nicotinic acid in plasma and an impaired control of FFA release and VLDL secretion

It may seem practical to advise patients with dif*ficulties in taking nicentrol* tablets in the middle of the day to divide the daily dosage into two doses instead of three This study has however *demon*strated that this change in the administration of the *drug markedly reduces the serum lipid lowering ef*fect

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Changes in the Adrenergic Control and the Rate of Lipolysis of Isolated Human Adipose Tissue during Fasting and after Re-feeding

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ABSTRACT The influence of changes in nutritional state on lipid mobilization has been investigated using subcutaneous fat portions removed from 8 obese patients submitted to fasting for one week The maximal rise in the tissue level of cyclic AMP (cAMP) and the rate of glycerol release were determined when adipose tissue was exposed to noradrenaline (6×10^{-6} mol/l) isopropylnoradrenaline (6×10^{-6} mol/l) or none of the agents (basal medium) Fasting resulted in significant increases in the tissue level of cAMP *and the rate of lipolysis in adipose tissue unexposed to* lipolytic agents Noradrenaline decreased the level of cAMP in contrast to isopropylnoradrenaline, and significantly inhibited the rate of lipolysis during fasting These noradrenaline effects were abolished by the simultaneous presence of phentolamine (13 mmol/l) in the incubation medium Re feeding with Fertitene® for one day resulted in a diminished rate of basal lipolysis, whereas the cAMP level was unaffected The response of the cAMP accumulation to isopropylnoradrenaline was further augmented by re feeding Noradrenaline produced a significant rise in the level of cAMP and significantly stimulated the rate of glycerol production It is concluded that the nutritional changes are of significance for the adrenergic regulation of lipolysis as indicated by the response of cAMP to the catecholamines In pharmacological terms *fasting for one week resulted* in increased α as well as β adrenergic responsiveness Increased basal lipolysis during fasting may be related to an increased level of cAMP or a direct activation of lipase

Whereas a variety of hormones stimulate lipolysis in rat adipose tissue only catecholamines have a pronounced lipolytic effect in human adipose tissue (6) *In adipose tissue of man (7 29 30) and hamster* (20) there is conclusive evidence for the presence of both α and β adrenergic receptors rat swine dog and guinea pig have only β sites (8) In human adipose tissue α adrenergic stimulation inhibits lipolysis (7 29 30) probably because of a decreased production of cyclic AMP (cAMP) (8 17) whereas β receptor stimulation accelerates lipolysis owing to an increased production of cAMP (7 8 9 14) This dual mechanism of the catecholamines observed in vitro might well be of significance for the liberation of energy from adipose tissue in man

We have previously observed that noradrenaline has an antilipolytic effect in human subcutaneous adipose tissue obtained from obese subjects submitted to prolonged fasting (21) The present study was undertaken to explore further the influence of nutritional factors on the regulation of lipolysis by human adipose tissue Of special interest was the question whether changes in lipolysis induced by different nutritional states could be related to changes in the tissue level of cAMP

METHODS

Patients

The material comprised 8 females referred to the Medical Clinic because of obesity They showed no evidence of hepatic renal or endocrine disorders and had normal glucose tolerance and normal serum lipids Patient 5 suffered from mild hypertension and epilepsy treated with 0 05 g chlorthalidone and 0 4 g phenytoin respectively per day All were on an unrestricted diet before the study None of them had participated in attempts at weight reduction in the 3–4 months prior to the study Relevant clinical data of the patients are presented in Table I

Table 1 *Clinical data of eight obese females submitted to total starvation for one week followed by re feeding with Meritene® for one day*

Pat no	Age (y)	Height (cm)	Weight kg	Weight % of normal	Weight loss during starvation (kg)	Mean fat cell weight (×10⁻⁹ g) Before starvation	Mean fat cell weight (×10⁻⁹ g) During starvation	Intake of Meritene® during re feeding (kJ)
1	23	150	147 0	308 8	6 5	682	672	
2	57	158	104 7	169 7	4 7	900	915	
3	26	163	92 5	163 1	3 4	815	874	
4	38	170	127 0	197 2	4 0	857	824	1 200
5	35	168	97 0	154 0	6 5	632	505	1 100
6	28	173	109 7	171 2	5 8	662	787	900
7	35	172	131 3	170 4	7 2	656	837	700
8	34	163	93 8	156 6	7 2	661	743	900
Mean±S E	35±4	165±3	110 3±6 6	186 4±18 1	5 7±0 5	733±38	770±46	1 000±100

Experimental design

The patients were weighed on standard scales accurate to within 0 1 kg The degree of overweight was calculated from the tables of the average weight of a normal population computed by Documenta Geigy (11) During the pre fasting period they were kept on the general hospital diet containing 8 500 kJ (2 000 kcal) per day After one night of fasting the first fat biopsy specimen was taken at 8 a m and followed by a fasting period of seven days During this period the patients received only calorie free liquids and multivitamin capsules No complications were observed during the starvation period except that mild hypopotassaemia developed in two patients during the first three days of starvation This was easily corrected with oral supplements of potassium chloride At 8 a m on the eighth day of fasting the second fat biopsy was performed Five patients (nos 4–8) were then allowed free intake of Meritene® (Cortec Denmark) until 8 p m on the eighth day Thereafter the patients were again fasting and a third fat biopsy was performed on the following morning at 8 a m Thus the fasting period was 12 hours before the first and third biopsy The dry milk powder product Meritene® consists mainly of carbohydrates (lactose) and proteins 100 g contains 1 500 kJ (360 kcal) 33 g protein 0 7 g fat and 58 4 g carbohydrates It was given as a water solution (100 g/l) Details of the procedure were explained to each individual and informed consent was obtained The biopsy procedure has been approved by the Ethical Committee of the Karolinska Institute

Biopsy procedure

Approximately 3 g of subcutaneous adipose tissue was excised from the upper lateral part of the thigh which was anaesthetized with prilocaine chloride as described in detail previously (3) The pre fasting and re feeding biopsy specimens were taken from the left and the fasting biopsy specimens from the right thigh

Incubation procedure

Adipose tissue was divided into portions of approximately 50 mg each pre incubated for 30 min in Krebs Henseleit bicarbonate (KHB) buffer containing 40 mg/ml dialyzed bovine serum albumin (Armour Pharmaceutical Comp Eastbourne England Lot No R970) and then incubated in 1 ml of medium of the same type as above for 2 hours After incubation two aliquots (0 1 ml) of the medium were removed for glycerol determination as described by Wieland (27) and modified by Chernick (10) The significance of the use of glycerol release as an indicator of the rate of lipolysis (4) and the influence of local anaesthetic agents on lipolysis (3) have been discussed previously The pre incubation and incubation procedures have been described in detail (28)

The intracellular cAMP concentration was determined in adipose tissue pre incubated for 30 min in KHB buffer pH 7 4 containing 10 mmol/l theophylline (ACO Sweden) and then incubated for 10 min in a fresh medium of the same type Albumin was not present in these experiments since it has been shown to interfere strongly with the assay for cAMP (5 23) Incubation and pre incubation were carried out in glass vessels with 50 ml of medium per g of adipose tissue From several experiments it was ascertained that the maximal tissue level of cAMP occurs after exposure for about 10 min to adrenergic stimulating drugs (2) At this time the production and degradation of tissue cAMP were unaffected by increased intracellular accumulation of free fatty acids due to the omission of albumin from the buffer since basal as well as stimulated lipolysis of human adipose tissue is almost undetectable at 10 min of incubation (7) The amount of adipose tissue obtained from one biopsy meant that the tissue samples used for a single incubation had a wet weight of only 50–75 mg As it was frequently not possible to measure cAMP when tissue pieces with a wet weight of less than 100 mg were analysed without prior incubation (unpublished observation) it was necessary to add the phosphodiesterase inhibitor theophylline to the buffer in order to boost the cAMP level so that reproducible experiments could be performed

Agents added in vitro were phentolamine HCl (Ciba Switzerland) noradrenaline bitartrate (Astra Sweden) and isopropylnoradrenaline CHl (Winthrop England) All agents were dissolved in water and added to the incubation medium in portions of 0 1 ml or directly dissolved in

Table II *Tissue cyclic AMP (cAMP) and lipolysis by subcutaneous adipose tissue obtained from 8 obese females before and during total fasting*

Adipose tissue was obtained before and during total fasting for one week Pre incubation and incubation were performed as stated in the text In the absence and presence of 6×10⁻⁶M noradrenaline (NA) or isoprenaline (ISNA) Tissue cAMP was determined at 10 min and glycerol release at 2 h of incubation Each value represents the mean of 4–6 determinations I Initial F fasting

Pat no	cAMP (pmol/10 cells) Basal I	F	NA I	F	ISNA I	F	Glycerol release (μmol/10 cells) Basal I	F	NA I	F	ISNA I	F
1	1 445	2 674	1 955	1 491	5 6[illegible]	7 545	11 3	17 9	15 6	7 3	25 0	16 5
2	916	1 453	1 911	1 018	5 573	12 163	18 0	36 [illegible]	29 8	18 4	45 3	40 5
3	975	1 320	1 473	1 145	3 679	6 365	10 3	19 5	10 3	13 4	21 0	21 5
4	583	1 075	778	975	2 916	3 789	11 4	38 0	17 1	14 6	27 7	31 7
5	636	654	1 189	323	12 473	33 533	4 7	20 1	9 4	5 [illegible]	16 7	20 0
6	497	2 097	1 271	1 393	3 554	10 483	7 3	15 1	12 6	11 4	21 [illegible]	17 1
7	495	1 606	1 078	1 479	5 688	18 583	[illegible] 1	13 [illegible]	3 7	9 4	8 5	14 0
8	686	1 8 8	1 251	1 293	8 565	13 878	4 8	12 8	8 0	10 7	19 1	16 8
Mean+	779+	1 58[illegible]+	1 357+	1 127+	5 996+	13 2[illegible]4+	8 7+	21 6+	13 3+	11 3+	23 1+	[illegible] 3+
S E	114	216	146	135	1 11[illegible]	3 550	1 8	3 5	[illegible] 8	1 5	3 8	[illegible] 7
p	<0 005		NS		<0 0[illegible]		<0 005		NS		NS	

the incubation medium They were added in final concentrations known from previous studies (12 30 31) to give a maximal effect on the rate of lipolysis

All incubations and pre incubations were carried out at 37°C in a water bath cycling at 40/min Air was used as gas phase

Determination of cell size

Two fat specimens weighing about 30 mg each were used for determining the cell diameter according to the procedure developed by Sjostrom et al (25) 100 cells were measured and the mean cellular triglyceride content was calculated from the average diameter and the S D of the diameter according to the formula developed by Hirsch and Gallian (19) The number of fat cells incubated was calculated using the mean cellular triglyceride weight and the total triglyceride content of the fat portions

Assay of intracellular cAMP

cAMP was determined by a modification of the protein binding method of Gilman (15) as described in detail elsewhere (2 5) Approximately 50 mg of adipose tissue were used for the assay cAMP binding protein and [³H]-adenosine 3 ,5 monophosphate cyclic ammonium salt (spec act. 15 mCi/mmol) were obtained from Boehringer Mannheim s kit assay for cAMP (Cat No 16 89) Protein kinase inhibitor was prepared by the method of Appleman et al (1) as modified by Gilman (15)

Statistical analysis

The values are given as the mean and the standard error of the mean (S E) Significance of the differences was calculated by Student s paired or unpaired *t* test

RESULTS

In the 8 obese females submitted to one week of fasting the loss of body weight ranged from 3 4 to 7 2 kg (Table I) the mean daily loss being about 0 8 kg No significant changes were observed in the fat cell weights during fasting (Table I) It was surprising that patients allowed free intake of Meritene® during re feeding for one day had a mean intake of only 1 000 kJ (240 kcal) (Table I) This could be explained by their reluctance to drink more Meritene® because of its flavour

The in vitro changes of intracellular cAMP level and the rate of lipolysis by subcutaneous adipose tissue obtained from the whole group before and during fasting are shown in Table II Before starvation the addition of 6×10⁻⁶ mol/l isoprenaline (ISNA) an almost pure β-adrenergic agonist increased the mean rate of the glycerol release from 8 7 μmol/10 cells/2 hours under basal conditions to 23 1 μmol/10 cells/2 hours ($p<0\ 001$) The mean tissue cAMP content before fasting increased from 779 pmol/10⁷ cells under basal conditions to 5996 pmol/10 cells in the presence of ISNA ($p<0\ 005$) During starvation the mean basal glycerol release was significantly ($p<0\ 005$) increased to 21 6 μmol/10 cells compared with the pre fasting release and could not be further increased by the addition of ISNA The basal level of cAMP during fasting (mean 1 58[illegible] pmol/10⁷ cells) was twice as

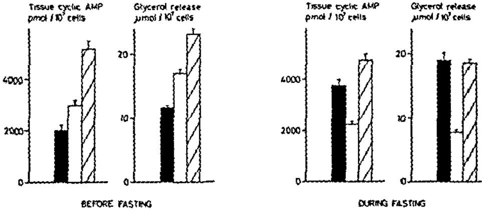


Fig 1 Effect of phentolamine on the noradrenaline (NA) induced changes in tissue cyclic AMP and glycerol release before and during fasting Subcutaneous adipose tissue was removed from patient 1 before and during fasting for 7 days Adipose tissue was pre incubated and incubated as stated in the text under basal conditions (■) with 6×10^{-6} mol/l NA (□) or with NA together with 13 µmol/l phentolamine (▨) Tissue cyclic AMP was determined at 10 min and the glycerol release at 2 hours of incubation (mean ±S E $n=5$–6 incubations)

high as before starvation and this difference was statistically significant ($p<0\ 005$) During starvation ISNA significantly increased ($p<0\ 02$) the mean tissue content of cAMP to 13 244 pmol/10^7 cells which was more than twice as high as the level before starvation ($p<0\ 02$) In contrast the rate of lipolysis induced by ISNA was similar in the pre fasting and the fasting state Before fasting 6×10^{-6} mol/l of noradrenaline (NA) significantly increased the mean tissue concentration of cAMP by 75% ($p<0\ 001$) and the mean glycerol release by 50% ($p<0\ 01$) compared with the basal values During starvation NA induced a significant decrease in mean glycerol release by 50% ($p<0\ 01$) as well a significant diminution in the tissue level of MP by 30% ($p<0\ 01$) There was no statistical correlation between the augmentation of basal tissue level of cAMP and the increase of the tissue cAMP response to ISNA that was induced by fasting Nor was there any quantitative relationship between the increment in the basal level of tissue cAMP and the enhancement of basal lipolysis during fasting in this group of individuals Before starvation the addition of phentolamine to NA containing incubation medium significantly potentiated the stimulatory effect of NA on the lipolysis by 40% ($p<0\ 001$) (Fig 1) Phentolamine also significantly augmented the NA induced rise of the intracellular level of cAMP by 80% before fasting ($p<0\ 001$) (Fig 1) During starvation the addition of phentolamine fully abolished the inhibitory effect of NA on the glycerol release ($p<0\ 001$) (Fig 1) *Phentolamine also completely* counteracted the NA induced decrease of the tissue cAMP level during fasting ($p<0\ 001$) (Fig 1) The concentration of phentolamine used in these experiments (13 µmol/l) had no effect on the basal glycerol release and the basal tissue level of cAMP either before or during fasting (data not shown)

In uncharted experiments the addition of 11 mmol/l glucose to the incubation medium did not prevent the antilipolytic or the tissue cAMP decreasing effect of NA during starvation Furthermore glucose had no significant effect upon the basal NA induced or ISNA induced tissue levels of cAMP in the fasting or pre fasting state The main effect of glucose in vitro was a rise in the basal glycerol release before and during fasting

Fig 2 shows the changes in tissue cAMP and lipolysis by subcutaneous adipose tissue obtained from 5 subjects (nos 4–8) before fasting during *fasting and after re feeding After re* feeding the basal glycerol release was significantly reduced ($p<0\ 025$) to approximately the same rate as before starvation The addition of ISNA after re feeding significantly increased the rate of glycerol release ($p<0\ 001$) to the same as that of the ISNA induced glycerol release before and during fasting After re feeding the ISNA induced cAMP level was 80% higher than during fasting ($p<0\ 05$) and three times higher than before starvation ($p<0\ 05$) The basal concentration of tissue cAMP was of the same magnitude after re feeding as during fasting and more than twice as high as the pre fasting value

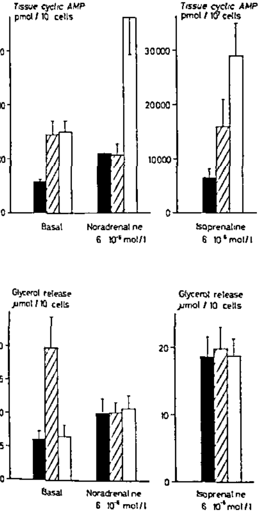


Fig 2 Metabolism of human adipose tissue during pre fasting fasting and after re feeding Subcutaneous adipose tissue was removed from 5 patients (nos 4–8) before fasting (■) during fasting for 7 days (▨) and after one day of re feeding subsequent to the fasting (□) Adipose tissue was pre incubated and incubated as stated in the text under basal conditions and in the presence of 6×10^{-6} mol/l noradrenaline or isoprenaline Tissue cyclic AMP was determined at 10 min and glycerol release at 2 hours of incubation (mean±S E) In the individual experiments 4-6 fat portions were used for each type of incubation The statistical symbols are for comparison of the basal values with the noradrenaline or isoprenaline induced values (*** $p<0.001$ *** $p<0.01$ * $p<0.25$ * $p<0.05$ NS=not significant)

($p<0.01$) After re feeding the response to NA was normalized with accelerated glycerol release ($p<0.025$) and elevated tissue level of cAMP ($p<0.05$) compared with the basal values

DISCUSSION

Catecholamine induced lipolysis

The present observations that NA had an inhibitory effect on the lipolysis induced by total starvation and that phentolamine invariably counteracted this antilipolytic effect are in full agreement with our previous findings (21) Furthermore the changes in the intracellular concentration of cAMP in adipose tissue exposed either to NA alone or to NA plus phentolamine run parallel to the changes in the rate of lipolysis Thus the results provide evidence for the hypotheses that an increased α adrenergic responsiveness of human subcutaneous adipose tissue exists during starvation and that increased α adrenergic responsiveness is related to a lowering of the cAMP in human adipose tissue The ISNA induced rise in the cAMP concentration was significantly more prominent ($p<0.02$) during than before starvation thus reflecting also increased β adrenergic responsiveness during starvation Decreased phosphodiesterase activity as the main reason for the augmented response of cAMP to ISNA seems less probable since there was no correlation between the increments to the basal level of cAMP and to the ISNA induced level that were obtained by the fasting state The mechanisms behind these alterations in the responsiveness to the catecholamines during starvation are not known Intracellular acidosis could contribute since it has been shown that the omission of basic ions from the incubation medium increases the α adrenergic responsiveness of human adipose tissue (24) A lack of glucose per se does not seem to be the main reason since the addition of glucose in vitro did not normalize the catecholamine induced changes in the intracellular cAMP level On the other hand a small caloric intake of carbohydrates and proteins restored the lipolytic effect of NA at least partly Although a decrease in the activity of the cAMP phosphodiesterase by re feeding cannot be excluded it seems unlikely since no increase in the rate of basal cAMP level occurred compared with the fasting level The significantly higher rise ($p<0.05$) in the cAMP level in the presence of ISNA observed in re feeding when compared with fasting should rather be regarded as a further increment of the function of the β adrenergic receptors

After re feeding NA significantly increased the tissue level of cAMP ($p<0.05$) and the rate of lipolysis ($p<0.025$) Whether this was due to a

normalized α effect or whether the α effect was increased but less than the β effect is not known Anyhow the balance between the α and β receptor sensitivity was restored by re feeding Since there was no corresponding increase in the ISNA induced rate of lipolysis during fasting and re feeding the findings are compatible with the idea that the hormone sensitive triglyceride lipase or some other step below the cAMP level would be rate limiting for the maximal lipolysis

It is not likely that cAMP from the stroma cells significantly influenced the findings with NA and ISNA induced cAMP levels in the different nutritional states Firstly we have recently observed (2) that in human adipose tissue in vitro there is a strong positive relation ($r=+0\ 84$ $p<0\ 001$) between the glycerol release and the tissue level of cAMP in the presence of ISNA Secondly the findings with NA on tissue cAMP and glycerol release were uniform in the pre fasting the fasting and the re feeding state

Basal lipolysis

In adipose tissue not exposed to catecholamines there was an almost threefold increase in the rate of lipolysis during starvation which confirms previous observations by us (21) and others (14 16 22) This stimulated lipolysis may be due to a direct activation of triglyceride lipase or be secondary to a simultaneous rise in the intracellular cAMP level The increments in the basal lipolysis and cAMP however were not well correlated in these experiments and re feeding resulted in inhibition of the rate of glycerol production without concomitant changes in the cAMP level From this it might be assumed that basal cAMP level is of no importance for the rate of lipolysis under any condition or only during fed and fasting states We have recently found (2) that no correlation exists between the cAMP level and basal lipolysis when calculations were performed on the data from adipose tissue of 22 donors Still the possibility exists that within an individual there is a positive and direct relationship between increments in the basal cAMP level and in the basal lipolysis The lack of correlation between the two parameters during re feeding may thus be due to alterations in the sensitivity of the lipolytic system to the cAMP level It is also possible that cAMP from the stroma cells might have influenced the basal tissue levels of cAMP

Effects of carbohydrates on lipolysis

It is well known that glucose in vitro stimulates lipolysis by adipose tissue but the mechanism behind this phenomenon is not known In this study the main lipolytic effect of glucose was a rise in the basal glycerol release before as well as during fasting Glucose had no effect on tissue cAMP before or in the fasting state This supports the theory that the localization of the lipolysis promoting effect of glucose in human adipose tissue is close to hormonal sensitive lipase as discussed earlier (13 18 26) The induction of a fed state in vivo by administration of Meritene® resulted in changes different from those obtained with glucose in vitro Thus the rate of lipolysis was lower in adipose tissue removed after re feeding compared with fasting whereas the levels of cAMP did not differ It is possible that the hormonal changes induced by re feeding such as depressed glucagon or increased insulin secretion are responsible for these alterations in the rate of lipolysis which take place without concomitant changes in the cAMP level

It is apparent that the metabolism of human adipose tissue is very sensitive to changes in nutritional conditions Fasting for one week increased basal lipolysis maximally (i e the glycerol release could not be accelerated by ISNA) Normalization of the basal lipolysis and restored response to catecholamines was obtained with re feeding for only one day with such a low caloric intake as 1 000 kJ (240 kcal)

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Extreme Elevation of Transaminase Levels in Acute Heart Disease—a Problem in Differential Diagnosis?

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ABSTRACT Five patients admitted to the Coronary Care Unit at the Department of Medicine Serafimer lasarettet who developed extreme elevation of trans aminase levels, are discussed in terms of problems in differential diagnosis All five had manifest right ventricular failure on admission and four also had hypotension Three of the patients died, two sur vived The three post mortem examinations showed extensive infarctions of the left ventricle and septum The two survivors had had a prolonged ventricular tachycardia and a probable silent infarct, respective ly It is concluded that the extremely high trans aminase levels sometimes seen in acute cardiac disease are predominantly due to sizeable amounts released by the liver as a result of central necrosis of the liver cells The probable prerequisite for the development of central necrosis of the liver in acute cardiac disease is usually the combination of right ventricular failure and hypotension which in turn are most often due to extensive left ventricular infarcts

Since la Due et al (10) first measured GOT in con nection with heart infarction in 1954 enzyme assay has been of great value in diagnosing this condi tion Excellent correlation has been demonstrated between GOT values and diagnoses verified by clinical and ECG observations (7) It is now routine at most clinics in this country to measure aspartate aminotransferase (ASAT previously GOT) ala nine aminotransferase (ALAT previously GPT) lactate dehydrogenase (LD previously LDH) in cluding the thermostabile fraction (LD_1 previously LDH_1) and sometimes creatine phosphokinase (CPK) *At the Medical Department at Serafimer* lasarettet a diagnosis of acute heart infarction requires that two of the following criteria might be met typical symptoms ECG changes of the type associated with acute heart infarction the enzyme pattern of acute heart infarction or the identification at autopsy of myocardial necrosis of an age corresponding to the date at which symptoms were first observed (8 20)

It is difficult at times to interpret enzyme values when in addition to ASAT (GOT) an increase in ALAT (GPT) is observed In such patients the diagnosis must be based on a typical CPK or α hydroxybutyrate dehydrogenase (HBD) curve or on typical ECG findings Every now and then patients are seen at heart infarction clinics with extremely high transaminase values The following case his tories illustrate the discussions as to differential diagnosis which may concern such patients

LABORATORY METHODS

Routine determinations were carried out according to a procedure which has been described previously (24) for GOT GPT LDH LDH_1 and CPK Our laboratory changed the methods of analysis and the units of measure *ment for transaminases on Nov 18 1974 and those for* LD and CPK a week later The blood samples have been analyzed in accordance with previous procedure in a Reactions Rate Analyzer (LKB 8600) Reagents for ASAT and ALAT were supplied by J T Baker Holland The activity determinations for the enzymes are now carried out at 37 C instead of at 35 C as previously The present and former denominations and reference values are shown in Table I One patient (no 3) was treated at the Depart ment shortly after the change in transaminase deter minations

THE PATIENTS

The subjects were five patients treated at the Medical Department Serafimerlasarettet who developed ex tremely high transaminase values during their stay in the hospital Age sex and pertinent laboratory data are given in Table II

Table I *Der t i t s ar d referc tcc val tes f r tl e en smes*

Prev ous		New	
GOT	< 40 U/l	ASAT	< 0 70 μkat/l
GPT	< 40 U/l	ALAT	< 0 70 μkat/l
LDH	<350 U/l	LD	< 7 2 μkat/l
LDH	< 75% of LDH	I D	<70% of I D
CPK	<100 U/l	CPK	< 1 7 μkat/l

Pa en 1

A 54 year old marr ed male w th three ch ldren who h d been generally healthy unt l J n 1974 when dm tted *w th substernal che t p ms of 11 hours durat n* The ECG showed ventr cular tachycard a w th a pulse rate cf more than 150 m n Immed ate electroconver on was accompl shed w th a retu n to s nus rhythm On dm s n he showed signs of both left and r ght s ded fa lure H s central venous p es ure was 18 cm H_2O Foll wing the in t al electroconvers on h s ECG d splayed s neg t ve T wave corresp nd ng to the anter r w ll of the left ventr cle Th returned to normal after one week Very h gh transam nase levels were detected on the second and th rd days w th the follow ng max m m v lues GOT *3 130 U/l GPT 7880 U/l LDH 6050 U/l* The pat ent was d scharged after 14 days n good cond t n at wh ch t me GOT was 4(U/l and GI T I(8 U/l H s rel t ve heart volume was 500 *ml/m²* BSA

The pat ent was readm tted after ne month for s bse quent l ver te ts at wh h t me the follow ng re ults were recorded GOT 44–42 U/l GPT 47–80 U/l I DH 290–375 U/l b l rub n 0 4–0 7 mg 100 ml alk l ne ph ph a e 34–28 U/l CPK 51–64 U l pro hromb n ndex (PTB) 54–38% Au ant gen nega ve The I rom ulphale n (BSP) test was abnormal w h 17 retent n of the 5 m nute *value X ray exam na on of the g l bladder revealed n* abnormal t e There wa n palpable enlargement of he l ver Ergometry was carried out and the max mum capac was found to be 120 W No arrhythm as appeared *was not pos ble to evaluate the ST segment a the* l ent was tak ng d gital

At fo low ups n 1974 the pat ent st ll had ev dence of l ver changes w th a GOT of 65 70 and GI T of 136–108 U/l

Pa ent 2

A 54 year old d v ced male l v ng al ne wh had ex per enced a ca d ac nfarct n n 1970 In March 1973 he was seen at Seraf merlasa e e w th abd m nal pa n and a fever of 40°C H pr ma y cond n w fe t t be cholecyst t s and ant b ot we c adm n e ed De p te this the h gh fever pers ed and he p t ent developed s gns of *r gl ven cul fa lu d I* k Beca e f the *marked elevat on of* tu an am n c l e s—G O I up 10 000 U/l—he was tran ferred a w d f nf t d seases on susp c on of hep t H we er t e ans am nases returned to no rmal relat vely q kly No n *fect ous etiology was establ shed On h o her hand* s gns of decompensat c n pr marily l f ded fa lu developed

In Dec 1973 the p t ent was readm tted w th a com pla nt of abd om nal p n and dy pnea The t n am na es were normal but the BSP test showed a re en ion of 31% of the 5 m nute v lue I TB was 47–68%

The p t ent w s t ken ll ag n n the beg nn ng of Feb 1973 w th abd m nal pa n and dyspnea The trans am n s es were n w n rmal but the BSI te t sh wed *delay w th a retention of 31% of the 5 m nute value* PTB was 47–68% At the end of Feb 1973 during a short trip abr ad he exper enced fat g e nau ea and dyspnea He ret rned to Sweden and on a r val at Seraf mer lasa ettet w s found t be dyspne c w th s gn of pul monary st s nd peripheral edem H s tempera ure varied between 38 and 39°C GOT was 600 U/l GPT 1700 U/l b l rub n 3 mg/100 ml and amylase 580 U/l The tr n m n es ret rn d t norm l n about one week Treatment w s st rte l w th d git l s nd d uret s

The pat ent was dm tted ag n n Apr l 1974 w th *che t p n shortnes of breath swelling of the legs* and vom t ng He w s n con de able d tress nd was found t have cyan t c l ps and asc tes O er a 3-day pe od GOT was 105 170 and 71 U/l and GPT 11 65 and 78 U/l I D 575 U/l HBD 40% bil rub n 1 5 mg % alkaline ph sph tases 23 U/l and amyl se 647 U/l The ECG revealed nf ct ch nges wh ch rema ned unal ered f r 3 days The p t ent s cond t on was judged t be p m rily due to severe card ac decompensat on together w th p n creat t s and he was treated w th parenteral nutrit on He d ed fter 3 d ys in the hosp t l

At a top y the he rt we ght was 6 0 g All chambers of the heart were markedly d lated The valves showed no al erat on The c ronary vessels d pl yed pronounced thicken ng f the w lls s a re ult of a te sclero s w th c n derable *reduct on in the lumen Exten ve* subend c rd al fibro s was found n the ent e w ll of the left ven ricl On sect on ng the ent re myoca d um was found to have nf rcts f vary ng ge The p ct re was dom n ted by old completely fibrot c re In add on a number of areas of granulat on me ng up to 3 cm n d meter were f und apparently r p esen ng 3–4 week old nfarcts I v dence f es ph g s was found wh le the p ncreas sh wed no p th l g l ch nges The s ze of the l ver was at the upper l n t of n rm al and the paren hyma sh wed m rked ev den e of sta The *color of the liver was s mewl t m e yel w h than* normal H t log cal exam na n revea ed ac e and chr n c sta w th central necr s of the n

Pa e 3

A 70 year ld m rr ed fem le w th a h t y of su pected stroke n 1971 In June 1974 she was fo nd to have

hypothyroidism which was treated with sodium levothyroxine At this time ECG was interpreted as being normal In Nov 1974 she was admitted with midthoracic pain In the six months prior to admission she had lost weight—nearly 10 kg On admission she was pale and had a pulse of 120 and a systolic BP of 100 mmHg The ECG revealed right bundle branch block

Shortly after admission she suddenly developed bradycardia and heart standstill with approximately 30 sec loss of consciousness Artificial ventilation and external heart massage were given together with i v methyl scopolamine to which the patient responded rather rapidly with sinus tachycardia and an increase in systolic BP to 110 mmHg However the BP fell again after a few minutes and the ECG showed total block Preparations were begun for using an artificial pacemaker but meanwhile she developed ventricular fibrillation for which defibrillation was carried out twice with satisfactory results After applying the pacemaker her systolic BP was approximately 100 mmHg During the next two days she had occasional episodes of tachypnea and blood streaked sputum Warfarin and heparin therapy were initiated on the basis of suspected pulmonary embolus She was mentally clear and the systolic BP varied between 95 and 100 mmHg However she experienced a sudden drop in PTB with hematemesis melena and a recurrent decrease in systolic BP to approximately 70 mmHg She received 2 l blood with favourable results on her general condition and BP During the next few days the patient felt well She was in sinus rhythm and thus the pacemaker could be removed Ten days after admission she again developed chest pains total block and asystole which could not be relieved

During her hospital stay her WBC rose to 24 600 On admission ASAT was 1 32 and ALAT 0 96 μkat/l ASAT reached its maximum value 14 4 μkat/l on the day after admission and ALAT 15 5 μkat/l on the third day after admission The maximum LDH value was 530 U/l while LDH remained normal throughout The maximum value for CPK was 404 U/l which was reached shortly before that for ASAT The values for alkaline phosphatases were 16 21 and 15 U/l Bilirubin was normal

At autopsy the heart weighed 350 g and showed no evidence of dilatation or hypertrophy The coronary vessels were markedly sclerotic and tortuous Old infarction alterations corresponding to 20% of the left ventricle were seen in the posterior wall of the ventricle Infarction changes some two weeks old and corresponding to approximately 75% of the left ventricle myocardium were seen in the septum from the apex up to the level of the valves and extending into the anterior and posterior walls Changes indicative of a new infarct were found in the anterior wall corresponding to approximately 20% of the left ventricle Some 85% of the myocardium of the left ventricle was judged to be infarcted There was no evidence of pulmonary edema or emboli There was a small stone in the gallbladder The pancreas showed no abnormal changes The liver was of normal size and displayed no evidence of fatty metamorphosis although stasis was apparent Histological examination of the liver revealed massive acute and chronic blood stasis with central necrotic changes in the acini

Patient 4

A 74-year-old married male with a history of relatively large alcohol consumption up to 4 or 5 years before admission He had been operated on for stomach ulcer approximately 20 years before the present illness He was admitted in June 1973 with left sided chest pains of sudden onset which were accentuated on inspiration and coughing

On admission he was in distress and pale His pulse was regular at about 100 and systolic BP 90 mmHg He showed signs of both left and right ventricular failure X ray examination of the lungs showed infiltration in the left lower lobe with the appearance of pleural pneumonia Some hours after admission the patient experienced a further drop in BP with severe respiratory distress and hemoptysis ECG showed changes indicative of a posterior wall infarct A pulmonary embolus was suspected with heart infarction or an acute abdominal condition as alternatives The patient was transferred to the CCU where the symptoms of both left and right ventricular failure increased during the following 24 hours and he developed oliguria Lung X rays three days after admission showed infiltration in the central portions of both lungs extending out to the lateral thoracic walls and large amounts of pleural fluid bilaterally Scintigrams of the lungs on the same day revealed limited diffuse absorption defects approximately 5 cm in diameter in both lungs The changes were considered to be results of possible multiple pulmonary emboli or pneumonic infiltration New X rays three days later showed increased pleural fluid and further extension of the infiltration His condition took a turn for the better when intensive failure therapy was introduced

The GOT level on admission was 34 U/l subsequently increasing to a maximum of 2200 U/l three days later with a decrease thereafter The initial GPT level was 280 U/l and reached a maximum of 1070 U/l The maximum LDH level was 4500 U/l The admission ECG showed signs which subsequently regressed of a posterior wall infarct The maximum WBC was 23 000 and the differential blood count showed a shift to the left The urine sediment showed 10–15 white and 15–25 red cells per field There was an initial increase in serum creatinine to 4 3 mg/100 ml with a subsequent decrease to 1 8 mg/100 ml prior to discharge The Weber test was positive on 3 occasions The clinical picture was interpreted initially as that of lung embolism

Two weeks after admission the patient developed new transient midchest pain with vomiting and nausea. After another ten days he was transferred from the CCU to an ordinary ward at which time he felt relatively well On July 24 he was found dead in bed

Autopsy revealed severe general arterial sclerosis thrombotic occlusion of the right coronary artery together with extensive myocardial infarcts of 3–4 weeks duration Pulmonary edema and a small stone in the common bile duct were also found but no emboli or infarcts in the lungs Histological examination of the liver showed the acinus structure to be preserved with signs of marked chronic stasis with some evidence of congestive fibrosis Microscopic examination of the heart showed confluent infarcts in the septum some fresh and some 10 days to 6–7 weeks old

Table II *Clinical and laboratory data on the patients*

Pat no	Age (y)	Sex	BP on admission (mmHg)	Maximum enzyme values				
				GOT (U/l)	ASAT (μkat/l)	GPT (U/l)	ALAT (μkat/l)	LDH (U/l)
1	54	♂	Undetectable	3 130		2 880		6 050
2	54	♂	Undetectable	6 000		10 000		?
3	70	♀	100/70		14 4		15 5	530
4	76	♂	90/60	2 200		1 070		4 500
5	86	♂	140/90	3 300		2 100		3 150

Patient 5

An 86-year old male who had received oral treatment for diabetes for 15 years He was admitted in Jan 1974 with a complaint of midchest pain of two weeks duration On the day before admission he had experienced increasing shortness of breath On admission he was in distress cyanotic pale and dyspneic His BP was 140/90 mmHg X rays showed an enlarged heart with marked stasis approaching pulmonary edema Some distension of the neck veins was also observed The liver was palpated some 5 cm below the costal margin in the mid clavicular line ECG showed regular sinus rhythm with a frequency of 95/min and left bundle branch block On the day after admission his GOT level was 3300 and GPT 2100 U/l The transaminases decreased gradually during hospitalization and had normalized before discharge Maximum LDH was 3150 U/l while LDH₁ remained normal throughout WBC increased to 9600 and ESR to 75 mm

The patient was given large amounts of i v diuretics with rapid relief of his cardiac decompensation The possibility that his antidiabetic drugs (chlorpropamide 37 5 mg and phenformin 100 mg daily) might be affecting the liver was discussed and they were discontinued One week later they were started again without any increase in the transaminases Bilirubin was normal Alkaline phosphatases were 49 U/l The BSP test showed increased retention with 26% of the 5 minute value after 45 min Electrophoresis showed a hypoalbuminemia without an increase in γ globulins A biopsy of the liver revealed no parenchyma cell damage The patient was discharged about one month after admission in good condition His transaminase levels were then quite normal

Approximately two months later he was readmitted with a complaint of fatigue nausea and vomiting and was found to be dehydrated He received parenteral fluids together with large amounts of oral fluids and recovered quickly ECG still showed LBBB The transaminase values were normal

DISCUSSION

Enzyme assays are now routine procedure in the diagnosis of acute cardiac infarct However a number of other factors can result in increased enzyme levels thus introducing diagnostic problems particularly if the clinical picture is not clear (6 14) In patients who display elevated levels of both ASAT (GOT) and ALAT (GPT) liver involvement should be suspected especially if the increases are very extreme (24)

It would appear that liver involvement is relatively common in connection with acute cardiac infarction In a recent study (25) more than half the patients with acute cardiac infarcts showed signs indicating a certain degree of liver involvement without any clinical evidence of right sided failure This was ascribed to possible subclinical right sided failure in these cases due to the fact that γ glutamyl transferase an enzyme which is specific for the liver was more commonly increased in cardiac infarcts with ECG evidence of right ventricular involvement than in infarcts of the left ventricle

Patients with extreme increases in enzymes—GOT and GPT values exceeding 1 000 U/l—present a special problem in differential diagnosis One g of heart muscle contains 156 000 U of GOT and 7 000 U of GPT (21) Although GOT activity in heart muscle is many times greater than that of GPT

ECG	Outcome
Ventricular tachycardia transient anterior T inversion	Still alive and well Moderately reduced liver function
Pathological Q wave posterior and ST elevation anterior	Deceased entire myocardium of the heart infarcted
RBBB	Deceased 85% infarction of left ventricle
Pathological Q wave posterior and right ventricular strain	Deceased extensive infarction of left ventricle
LBBB	Still alive and well Moderately reduced liver function

there is still an adequate source for an increase in GPT in sizeable cardiac infarcts The correlation between maximum GOT and GPT levels however is relatively poor (25) while good correlation between infarct size and peak GOT LDH and CPK levels has been demonstrated in a number of studies (1 11 12 16 22) According to Sobel et al (22) it would appear that the maximum levels of both GOT and LDH may be increased by added amounts from the inflammatory infiltrate in the heart which develops in cases of acute cardiac infarct Thus these maximum enzyme levels may not necessarily be in direct correlation with the amount of myocardium which has been infarcted On the other hand animal experiments (23) indicate that CPK levels are in direct correlation with the size of the cardiac infarct A convincing correlation between maximum GOT and CPK levels has not been demonstrated however which suggests that a certain amount of GOT may be put out by the liver (25) In a recent autopsy study Erhardt (12) was able to demonstrate good correlation between infarct size and GOT LDH and LDH_1 levels in patients without concomitant GPT increases On the other hand he found no correlation between maximum GOT levels and infarct size in patients with simultaneous GPT increases a situation which might occur if the GOT increases were partly due to a release of enzymes by the liver Maximum LDH and LDH_1 levels however were found to be well correlated with infarct size despite concomitant GPT increases

GOT and LDH can also increase in addition to GPT in acute and subacute damage to the parenchyma of the liver and in the liver as well the maximum levels of these enzymes are thought to be related to the magnitude of the disease process In liver disease the changes in GOT and GPT concentrations are similar although GPT is usually more pronounced (19) Especially high GOT and GPT values are seen in association with anoxic damage or toxic or infectious hepatitis values exceeding 1000 U/l frequently being seen with these conditions In acute necrosis of the liver consequent to ligation of the hepatic artery GPT values of 25 000 U/l may be seen (2) Diseases of the bile ducts with or without occlusion of the ducts produce transaminase elevations which ordinarily do not exceed 500 U/l In conditions involving chronic depression of liver function such as cirrhosis the GOT levels are only slightly elevated or may even be within normal limits In alcoholic hepatitis however higher GOT and GPT values are frequently seen but seldom exceed 500 U/l (2) In a recent study of 160 consecutive patients in Gothenburg (27) it was found that most patients with ASAT levels exceeding 500 U/l (normal level <17 U/l) had acute hepatitis circulatory disturbances (hypotension of long duration shock pronounced cardiac failure) with or without cardiac infarct or malignancies of the liver In acute hepatitis the increases in ASAT and ALAT were usually of the same magnitude However when the ratio of ASAT to ALAT exceeded 3 it was usually associated with liver malignancy or in a few patients with circulatory disturbances

All three deceased patients in our series had low BP on admission—patients 2 and 4 presenting with a clinical picture approaching preshock Autopsy revealed that almost all of the left ventricle was infarcted Autopsy surveys have shown that cardiac infarct patients who are in shock have larger infarcts than those who are not (3 11 12 15) According to Bang and la Due (5) the average maximum enzyme increase in cardiac infarct patients in shock is five times as high as in those not in shock

The patients in the present study who died were found to have moderate to pronounced stasis in the liver at autopsy Necrosis of the liver cells was also found on microscopic examination in two (nos 2 and 3) while patient 4 had an early fibrosis Killip and Payne (17) have observed

necrosis of the liver cells in 17 patients who had GOT elevations exceeding 500 U/l together with acute cardiac disease (infarct or failure) without primary liver disease Signs of right ventricular failure hypotension or shock were frequently seen in these patients prior to the increase in GOT Central necrosis of the liver cells was seen in the 8 autopsied patients

Bang et al (4) also found a correlation between the increase in GOT and the extent of central necrosis of the liver cells in patients with cardiac infarcts but they were unable to find any correlation between the size of the infarct at autopsy on the one hand and the GOT levels and extent of central necrosis of the liver cells on the other

The deceased patients in the present series displayed signs of right sided as well as left sided failure on admission Acute right sided failure frequently results in acute stasis of the liver which in turn may cause a necrosis of the liver cells In such patients hypotension may well intensify the effect of the stasis in the liver cells (13 17)

The survivors also showed signs of right ventricular failure on admission BP was too low to be measured in patient 1 whereas patient 5 had a fairly normal pressure In patient 1 the hypotension as well as the right ventricular failure were consequences of his ventricular tachycardia on admission It seems likely that the patient had had ventricular tachycardia for 11 hours and thus quite possibly right ventricular failure as well as hypotension It has also been reported that arrhythmias of long duration together with hypotension can result in central necrosis of the liver cells with marked increases in GOT levels (9) However electroconversion may have contributed somewhat to the increase in enzyme levels in our patients 1 and 3 Increases in one or more of the serum enzymes following electroconversion have been recognized previously (14 18 26 28) They are probably the result of transient changes in the skeletal musculature of the thorax and usually of moderate degree

Patient 5 in this series who also survived had a history of chest pain and increasing decompensation of two weeks duration On admission he had right ventricular failure but normal BP This patient had had an LBBB of several years duration making it impossible to diagnose his infarct by means of an ECG The normal LDH_1 values during his stay in the hospital would seem to indicate that there was no destruction of heart muscle at that time The increase in his enzyme levels would therefore appear to be due primarily to liver damage consequent to right ventricular failure Subsequent liver studies on this patient indicated only moderate functional loss with some increase in BSP retention together with a slight reduction in galactose tolerance Biopsy of the liver showed nothing remarkable although this does not exclude the possibility of parenchymal damage This patient illustrates the difficulties involved in differential diagnosis in the presence of massive transaminase elevations Nausea and confusion were the dominant features of the clinical picture Acute hepatitis or a toxic necrosis of the liver resulting from some medication was the preliminary diagnosis but subsequent developments made a diagnosis of silent myocardial infarction with right ventricular failure more probable

It has been shown that necrosis of the liver cells can heal and be replaced by fibrotic tissue (4) This mechanism may have taken place in our patients 4 and possibly 5

It is well known that moderate increases in GOT GPT and LDH can result from various more or less common factors (6 14) In addition to electroconversion in two of our patients (nos 1 and 3) anticoagulant therapy and consequent anticoagulant bleeding in one patient (no 3) may have caused a certain increase in enzyme levels but this probably was inconsequential in comparison with the enzymes released from the heart and liver In two patients (nos 3 and 4) pulmonary embolism was suspected but could not be verified at autopsy Hepatitis was considered as the probable primary cause for the increases in enzyme levels particularly in patient 2 but also in patient 5

All five patients reported here had manifest right ventricular failure on admission and in addition four had hypotension The right sided failure and hypotension in the three patients who died were due to extensive infarction of the left ventricle and the septum without demonstrable infarction of the right ventricle It has been shown (20) that there is a positive correlation between maximum GOT levels in cardiac infarction and pressure in the pulmonary artery It is believed that the most probable cause of the elevated pressure in the pulmonary artery is an increased filling pressure in the left ventricle In the three routine autopsies nitro-BT staining was not used in the histological

examination of the heart This technique provides better possibilities for delineating the extent of the infarction In an autopsy study of 84 deaths due to cardiac infarction where nitro-BT staining was used (12) it was found that the right ventricle was involved in approximately 40% of the patients Necrosis of the liver cells was found in 27% of all these patients and in 45% of those with right ventricular failure and with infarctions which also involved the right ventricle Consequently it may be suspected that the right ventricle was involved in our three patients who died However the massive left ventricular infarctions found in them would probably have been sufficient to induce right sided failure

In conclusion it would appear that the extremely high transaminase levels sometimes seen in acute cardiac disease are predominantly due to sizeable amounts released by the liver as a result of central necrosis of the liver cells The probable prerequisite for the development of central necrosis of the liver in acute cardiac disease is usually the combination of right ventricular failure and hypotension It is also possible that anoxic cell damage in the heart kidneys lungs intestines and muscles may contribute to the transaminase elevations in patients who have been in shock for any length of time (6)

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Hyperlipidaemia and Reduced Fibrinolytic Activity Associated with Thromboembolic Complications in a Family

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ABSTRACT Although not significantly correlated the occurrence of reduced fibrinolytic activity and capacity in hyperlipidaemics has often been described Hyperlipidaemia and reduced fibrinolysis might well enhance the possibility of thromboembolism Such a coincidence of risk factors has probably caused thrombotic disease in a family, as described in this case report and family investigation

Hypercoagulability is commonly accepted to precede thromboembolism and depends chiefly on increased synthesis of coagulation factors (8 15) A reduction in fibrinolytic activity might however also contribute to hypercoagulability in circulating blood With improved methods for recording fibrinolytic activity and capacity (17 18) an association has been shown between reduced fibrinolytic activity and thrombotic disease (9 13)

Recently Almer and Nilsson (2) reported a significantly reduced fibrinolytic activity in patients with diabetes mellitus Fibrinolytic activity was not however significantly correlated with hypercholesterolaemia and/or hypertriglyceridaemia Nevertheless in patients with hypercholesterolaemia the mean spontaneous fibrinolytic activity and in patients with hypertriglyceridaemia the mean fibrinolytic capacity were only two-thirds of the respective means in those with normal lipid values (7) The findings of hypertriglyceridaemia and reduced fibrinolytic capacity correspond well with the observations described below

CASE REPORT AND FAMILY INVESTIGATION

A 24 year-old man previously in good health was on Feb 9 1975 admitted to the Department of Neurology Ullevål Hospital On admission he had a paresis in his right arm and leg which had developed during the last four days Dysphasia was never observed (he is lefthanded) and the hemiparesis disappeared a few days after hospitalization EEG cerebral scintigraphy and cerebral angiography were normal on admission and so was the cerebrospinal fluid except for a slightly increased protein value (48 mg/100 ml normal values below 45) Routine coagulation tests revealed no signs of hypercoagulability A considerable hypertriglyceridaemia (fasting value 4 28 mmol/l normal range 0 40–1 70) and a moderate hypercholesterolaemia (308 mg/100 ml 7 98 mmol/l) were discovered however A diagnosis of a minor cerebral infarction in the left hemisphere was made and the patient was referred to the Haematological Laboratory for further investigations since inherited thromboembolic risk factors had to be suspected

Thus a younger brother had had a thrombus (at the age of 17 in 1972) in the right popliteal artery which had been removed by thrombendarterectomy One year later he was treated with heparin for a venous thrombus in his left leg On this occasion the routine coagulation tests were normal as was the serum cholesterol though triglycerides were not determined.

The parents and a sister were in good health but the father's sister had had a myocardial infarction in 1953 at the age of 40 Xanthomata were present but serum cholesterol was normal (19? 181 mg/100 ml−4 97−4 69 mmol/l) She developed a heart insufficiency and died 46 years old from a second myocardial infarction

As to acquired thromboembolic risk factors it should be mentioned that all family members were cigarette smokers but none of them were obese

METHODS

In all family members the fibrinolytic response to venous occlusion (=fibrinolytic capacity) was examined (twice) and compared with the fasting serum levels of blood sugar cholesterol and triglycerides The lipoprotein patterns on paper electrophoresis were determined and were compared with the individual fibrinolytic capacity A platelet adhesiveness test and an antithrombin III assay in plasma were also performed

Table I *Blood sugar and serum lipids (fasting values) lipoprotein pattern and fibrinolytic response to venous occlusion in the patient and his family*

See Methods for test references and normal ranges of values

	Patient (24 5 y)		Brother (20 y)		Sister (23 y)		Father (54 y)		Mother (51 y)	
Date of investigation (1975)	3 3	11 4	3 3	11 4	3 3	11 4	18 3	11 4	18 3	11 4
Serum glucose[a] (mg/100 ml)		88		93		86	102	90	87	93
Serum cholesterol[b] (mg/100 ml)		283		262		212	315	340	347	358
Serum triglycerides (mmol/l)		3 27		2 82		2 47	2 50	2 28	5 8[illegible]	4 7[illegible]
Type of lipoprotein pattern		IV		IV		N	IIB	IIB	IV	IV
Euglobulin lysis time (min)										
Before stasis	>120	>120	>120	>120	>120	>120	>120	>120	>120	>1[illegible]0
After stasis	>120	130	24	43	15	24	17	15	65	48
Fibrinolytic capacity[c]	R	R	N	R	N	N	N	N	R	R

[a] *100 mg/100 ml≈5 55 mmol/l*
[b] *100 mg/100 ml=2 59 mmol/l*
[c] *R=reduced N=normal*

Fasting serum glucose cholesterol and triglycerides were determined at the Central Laboratory Ullevål Hospital The methods used are described in the Oslo Study (11) Normal ranges glucose 70–115 mg/100 ml (3 9–6 4 mmol/l) cholesterol 150–350 mg/100 ml (3 9–9 1 mmol/l) *triglycerides 0 40–1 70 mmol/l* The latter two values *concern age groups 20–55 years*

Lipoprotein paper electrophoresis was performed at the Lipid Laboratory of the Medical Outpatient Clinic Ullevål Hospital The lipoprotein patterns were mainly typed in accordance with the classification of the WHO (19)

Antithrombin III concentration in plasma was measured immunologically by the Mancini technique (12) at the Immunohaematological Laboratory Ullevål Hospital Normal range 100–150% of normal plasma concentration

Platelet adhesiveness tests (6) were performed by *Dr J le at the Institute for Thrombosis Research Rikshospi t Oslo* Normal range 60–90%

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RESULTS

No abnormalities in platelet adhesiveness were found and the antithrombin III plasma concentrations were normal The other results are listed in Table I

The fasting serum triglyceride levels were increased in all family members but the lipoprotein patterns differed as did the fibrinolytic capacity However those family members who had experienced thrombotic disease (the patient and his brother) showed *lipoprotein patterns* (type IV) identical to that of their mother, and all three had reduced fibrinolytic capacity with the exception of *one normal reading Unfortunately serum lipid de*terminations were not performed on that occasion

DISCUSSION

Reduced fibrinolytic activity and capacity have been correlated with diabetes mellitus (2 3) and *obesity* (1 5) A negative correlation between fibrinolytic activity and serum *triglycerides* was found in a random sample of 788 men aged 54 (10) but the *correlation* was not significant if the effect of obesity was taken into account The *present* study suggests an association between hyper pre β *lipoproteinaemia* and *reduced* fibrinolytic capacity and possibly also between *increased serum triglycerides* and reduced fibrinolysis

Moderate lipid *reducing* diet did not decrease the serum triglyceride *level* in this case From April 24 1975 the warfarin treatment was therefore supplemented with clofibrate medication After five months medication and without *a contemporary* reduction in body weight the serum *triglyceride* level had *decreased* from 3 27 to 2 30 mmol/l but the patient still *demonstrated* a *pre β* lipoprotein pattern and the fibrinolytic capacity was *unchanged* (euglobulin lysis time after stasis still exceeded two hours) *If the plasminogen* activator content of the

vessel walls in this case were reduced due to an inborn defect this might possibly explain the unchanged fibrinolytic response to venous occlusion

Studies on the effect of alimentary lipaemia upon the release of plasminogen activators have shown that the increased release which occurs in response to venous occlusion remains unmodified (4 7 14) It also seems unlikely that the presence of increased concentrations of circulating triglycerides per se influences the assay of fibrinolytic capacity This assumption was supported by the fact that the patient s plasma euglobulin fraction used in the fibrinolytic capacity test (16) did not contain lipoproteins In accordance with this euglobulin lysis time after and prior to stasis remained unmodified when the increased plasma triglyceride and chylomicron fractions obtained after fat ingestion by a healthy volunteer were mixed in equal proportions with the plasma samples collected for the test (unpublished data)

In spite of numerous studies into the effects of plasma lipids upon fibrinolysis (5 7 14) the importance of concomitant occurrence of hyperlipidaemia and reduced fibrinolysis for the development of thrombotic disease is still unclear Therefore in order to obtain further information about the possible relationship between reduced fibrinolytic capacity and various risk factors for atherosclerotic disease co-operation has been established with the Oslo Study (11)

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Table I *Blood sugar and serum lipids (fasting values) lipoprotein pattern and fibrinolytic response to venous occlusion in the patient and his family*

See Methods for test references and normal ranges of values

	Patient (24 5 y)		Brother (20 y)		Sister (23 y)		Father (54 y)		Mother (51 y)	
Date of investigation (1975)	3 3	11 4	3 3	11 4	3 3	11 4	18 3	11 4	18 3	11 4
Serum glucose (mg/100 ml)		88		93		86	102	90	87	93
Serum cholesterol[b] (mg/100 ml)		283		262		212	315	340	347	358
Serum triglycerides (mmol/l)		3 27		2 82		2 47	2 50	2 28	5 87	4 77
Type of lipoprotein pattern		IV		IV		N	IIB	IIB	IV	IV
Euglobulin lysis time (min)										
Before stasis	>120	>120	>120	>120	>120	>120	>120	>120	>120	>120
After stasis	>120	130	24	43	15	24	17	15	65	48
Fibrinolytic capacity[c]	R	R	N	R	N	N	N	N	R	R

[a] 100 mg/100 ml=5 55 mmol/l
[b] 100 mg/100 ml=2 59 mmol/l
[c] R=reduced N=normal

Fasting serum glucose cholesterol and triglycerides were determined at the Central Laboratory Ullevål Hospital The methods used are described in the Oslo Study (11) *Normal ranges* glucose 70–115 mg/100 ml (3 9–6 4 mmol/l) cholesterol 150–350 mg/100 ml (3 9–9 1 mmol/l) triglycerides 0 40–1 70 mmol/l The latter two values concern age groups 20–55 years

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Moderate lipid reducing diet did not decrease the serum triglyceride level in this case From April 24 1975 the warfarin treatment was therefore supplemented with clofibrate medication After five months medication and without a contemporary reduction in body weight the serum triglyceride level had decreased from 3 27 to 2 30 mmol/l but the patient still demonstrated a pre β lipoprotein pattern and the fibrinolytic capacity was unchanged (euglobulin lysis time after stasis still exceeded two hours) If the plasminogen activator content of the

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A Comparison of Two Methods for Estimating Bone Loss

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ABSTRACT The presence or absence of osteopenia, as judged by routine X ray examination of the lumbar spine supplemented by X ray evaluation of calcaneus, has been investigated in 73 female subjects who also had photon absorptiometry done on calcaneus and antebrachium Comparison of the two methods reveals that there is no close relationship between the results Photon absorptiometry for evaluating bone mineral content has proven its precision and accuracy, while conventional X ray film evaluation of bone mineral seems to lack a proper foundation

Attempts to estimate the bone mineral content (BMC) from X rays have been made for several years In the daily clinical routine visual evaluation of lumbar spine X rays is the common procedure It is questionable however whether this much used method is sufficiently reliable i e whether its clinical benefit balances the considerable cost involved To evaluate this the method should be compared with measurements of the BMC obtained in a more precise manner

In vivo photon absorptiometry has become clinically practicable during the last decade (2 3 6) This method has an excellent precision and accuracy (2 6) for measuring local BMC Furthermore it can yield a useful estimate of total body calcium when applied to suitable bones (4 8 10) usually those of antebrachium So far the technique is available only in specialized centres with a research interest in calcium metabolism

It is reasonable to compare the common X ray film technique with the more sophisticated technique of photon absorptiometry As a preliminary communication we present here data concerning the presence of X ray osteopenia in female subjects with normal BMC values

MATERIAL

Seventy three females between 21 and 70 years (mean 43) participated in the study They were randomly selected among the hospital staff and out-of bed patients from the Departments of Otorhinolaryngology and Psychiatry Patients from the latter group were examined only if diazepam was the sole pharmacological treatment and no psychotic patients took part All 73 females fulfilled the following criteria No symptoms of digestive or renal diseases Serum creatinine value <14 mg/l No pharmacological treatment except diazepam No past or present treatment with contraceptive pills or hormonal substitution

METHODS

BMC was determined by direct photon absorptiometry on both forearms and both calcanei The extremity to be examined is fixed in a plexi glass container filled with distilled water (Fig 1) The source of radiation (25 mCi ^{125}I) and the detector are fixed in a holder on either side of the bones to be examined A mechanical scanner displaces the detector and the source perpendicular to the longitudinal axis of the bones The transmission of the photons through the bones depends on the bone mineral content

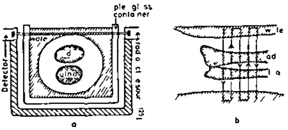


Fig 1 Principles in measurement of bone mineral content by direct photon absorptiometry on forearm *(a) Section* through system showing plexi glass container and U shaped holder with radioactive source and detector *(b)* System seen from volar surface showing scanning movements of source and detector

Table I *Distribution of BMC calcaneal values in females without and with X ray osteopenia of calcaneus in relation to corresponding normal mean (x) and S D*

		BMC values					
			Between (=normal range)				
No of pats	X ray osteopenia of calcaneus	Less than x−2 S D	x−2 S D and x−1 S D	x−1 S D and x	x and x+1 S D	x+1 S D and x+2 S D	More than x+2 S D
65	Absent	0	10	16	28	11	0
8	Present	1	2	3	2	0	0

which is directly related to bone calcium content (4) Because the linear attenuation coefficients of plexi glass distilled water and soft tissue are almost identical the measurement is independent of the amount of soft tissue Details of the procedure are published elsewhere (4 5 6)

X rays were taken with a 85 KV unit Whether or not osteopenia was present (i e decreased mineral content as seen in osteoporosis and osteomalacia) was evaluated by one of us (B D) who was unaware of the clinical findings Lateral projections of the lumbar spine and lateral plus axial projections of the calcanei were made according to the standardized procedure of the department Only two possibilities were given for the evaluation osteopenia absent and osteopenia present for each of the two localizations

RESULTS

The results are given in Tables I and II Calcaneus was used to compare the two methods reliability in stimating *local* calcium content out of the 73 sub ts eight persons (11%) had osteopenia of aneus

Antebrachium and the lumbar spine were examined to give an estimate of total body calcium Eleven subjects (15%) showed X ray osteopenia in spite of normal BMC values

DISCUSSION

If the results from the two methods correlated then the patients with X ray osteopenia should have low BMC values For the evaluation of *local* calcium content (calcaneus Table I) there was a slight but insignificant tendency in this direction inasmuch as six out of eight subjects with calcaneal X ray osteopenia had a BMC value below the normal mean For the evaluation of *total* body calcium (lumbar spine Table II) this tendency was not present

The precision of *local* BMC measurement is in the region of 2–4% (2 6) while Lachman (7) estimated that a bone loss of 30–60% was needed before the unaided observer was likely to be confident about bone rarefaction from X ray films

Total body calcium can be estimated from antebrachial BMC measurements with a standard error of estimation of 10–13% (4 8) but it has not been possible to estimate total body calcium from routine X ray methods due to the difficulties in quantitating the findings and the variation arising from minor differences in the radiological factors and in the amount of overlying soft tissue There

Table II *Distribution of BMC antebrachial values in females without and with X ray osteopenia of lumbar spine in relation to corresponding normal mean (x) and S D*

		BMC values					
			Between (=normal range)				
No of pats	X ray osteopenia of lumbar spine	Less than x−2 S D	x−2 S D and x−1 S D	x−1 S D and x	x and x+1 S D	x+1 S D and x+2 S D	More than x+2 S D
62	Absent	2	2	30	20	6	2
11	Present	0	4	3	3	0	1

fore it might seem that the common procedure of evaluating osteopenia from plain X ray films lacks a proper basis If X ray examinations are to be employed then more sophisticated methods are clearly necessary (1 9)

A prospective study on a larger number of subjects representing normal *and* pathological calcium metabolism would be of value to clarify the sensitivity and specificity of different X ray film evaluations of generalized osteopenia

ACKNOWLEDGEMENT

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Serum Glucose Determination with Dextrostix and the Eyetone Reflectance Meter

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ABSTRACT A simple modified procedure for the Dextrostix–Eyetone system has been evaluated in order to enable the system to measure the concentration of glucose in serum as well as in whole blood A reduction of the ordinary time of reaction on the Dextrostix from 60 to 45 sec gave serum glucose determinations by the Dextrostix–Eyetone system that correlated almost perfectly with those obtained by a specific conventional laboratory procedure Thus the coefficient of correlation was 0 99 and the regression line very close to the ideal line As the modification is very simple and does not involve any changes in the adjustment of the instrument, it is recommendable in all cases where only serum samples are available

Blood glucose determination by the Dextrostix–Eyetone system is a precise and reliable alternative to conventional laboratory methods (2) In the performance of the ordinary Dextrostix–Eyetone procedure a sample of whole blood is required the sample being transferred to the Dextrostix by means of a pipette or even more easily by dipping the strip into the sample (1) For many hospital and laboratory purposes serum is however a preferable medium to whole blood and furthermore serum may be stored deep-frozen for later analysis The practical value of the Eyetone measuring device would hence be considerably increased if it could also be applied to serum samples

The aim of the present investigation was to evaluate a modification of the current operating procedure for the Dextrostix–Eyetone system in order to enable the system to work with serum as a substrate

MATERIAL AND METHODS

Serum samples were obtained from patients submitted to glucose tolerance tests or insulin hypoglycemia tests for other reasons Hyperglycemic samples were also prepared by adding a concentrated aqueous glucose solution In a preliminary study 34 serum samples were analysed in duplicate by the conventional 60-second Dextrostix–Eyetone procedure As the results did not compare satisfactorily with those obtained by a glucose oxidase reference method (3) new series of experiments were initiated in order to investigate if changes in the time of reaction on the Dextrostix might improve the performance of the system A 15 second reduction of the time of reaction appeared to yield almost correct results Consequently 90 serum samples were analysed in duplicate by the reference method (3) as well as by the Dextrostix–Eyetone procedure allowing a 45 second time of reaction only Otherwise the measuring was done in close accordance with the operating manual Serum was applied to the reagent area of the strip by means of a pipette

Orthogonal regression analysis was applied to test the correlation between serum glucose concentrations obtained by the reference method and the Dextrostix–Eyetone procedure Analysis of variance was used for comparing the precisions of the methods The significance of differences between variances was tested by means of the F test P values less than 0 05 were considered significant

RESULTS

Fig 1 shows concentrations of serum glucose obtained by the Dextrostix–Eyetone system using a 60 second reaction time and the reference method The coefficient of correlation is 0 99 and the regression line $y=1\ 24x-4\ 0$ Fig 2 shows that when the time of reaction was reduced to 45 sec the coefficient of correlation was still very high (0 99)

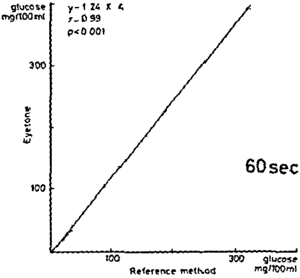


Fig 1 Correlation between the concentration of glucose in serum determined by the Dextrostix–Eyetone procedure (*time of reaction 60 sec*) *and a routine glucose* oxidase method Each point represents the mean of corresponding duplicate determinations $y=x$

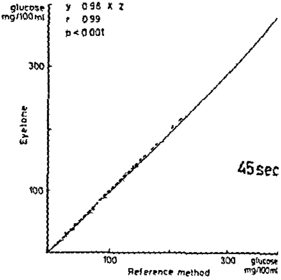


Fig 2 Correlation between the concentration of glucose in serum determined by the Dextrostix–Eyetone procedure (*time of reaction 45 sec*) *and a routine glucose* oxidase method Each point represents the mean of corresponding duplicate determinations $y=x$

and the regression line ($y=0\ 98x-2\ 0$) considerably improved

Tables I and II give data on the precision and variance of the reference method and the 45 second Dextrostix–Eyetone procedure Serum glucose concentrations determined by the Dextrostix–Eyetone procedure were on an average 3 2% lower than those obtained by the reference method The standard deviations and coefficients of varia were however almost identical (Table I) in the ranges of 10–100 100–200 and 300–400 g/100 ml of serum glucose the variances of the two methods compared well Within the range of 200–300 mg/100 ml the variance of the Dex stix–Eyetone procedure was however cantly greater than that of the reference In the material as a whole the variance

Dextrostix–Eyetone procedure was slightly but not significantly greater than that of the reference method (Table II)

DISCUSSION

Both Dextrostix–Eyetone procedures for serum glucose determination correlated almost perfectly with the reference method very high coefficients of correlation being obtained The conventional 60-second procedure was however not ideal for practical purposes since the slope of the regression line was well above one (Fig 1) A transcription of

Table I *Precision the Dextrostix–Ey system (time of rea sec) refer method*

	ce	tix
Mean (mg/100 ml)		
S D		
Coeff of variation		

II Varia time of r differe r Dextrostix–Eyetone sec) and the reference rum glucose

	o o ter	Comparison f varices
		01

the Eyetone read-outs would hence be required to get the correct concentration of serum glucose In contrast the 45 second procedure gave an almost perfect regression line (Fig 2) Within the 200–300 mg/100 ml range of serum glucose the variance of the Dextrostix–Eyetone results was significantly greater than that of the results of the reference method The variance was however not of a magnitude to compromise the practical useability of the system 95% of the results being within ±11 mg/100 ml of the correct serum glucose concentration

In conclusion a simple modification of the Dextrostix–Eyetone procedure was found to give readings of serum glucose concentrations that compared well with those obtained by a conventional laboratory method No changes in the adjustment of the instrument or its standard calibration were needed Alternating determinations of glucose concentrations in samples of whole blood and serum are hence possible The procedure can be recommended for use in general practice hospitals and laboratories whenever serum samples are available and rapid glucose determinations are needed

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Acta Med Scand 200 301–308 1976

Microcalorimetric Measurements of Heat Production in Human Erythrocytes

II Hyperthyroid Patients before during and after Treatment

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ABSTRACT Heat production has been measured in erythrocytes of 17 hyperthyroid patients both before treatment and when the patients had become clinically euthyroid The decrease in heat effect during treatment was significant The initial mean value was significantly higher than the corresponding value for normal subjects A good correlation was found between heat effect values and the clinical condition Measurement of heat production in erythrocytes can provide useful information about the peripheral effect of thyroid hormones

The mechanism of action of the thyroid hormones is still unknown and cellular physiology in connection with hyperthyroidism therefore continues to attract attention Glucose (27) and oxygen (1) consumption in erythrocytes of hyperthyroid patients have been found to be increased Incubation of erythrocytes from healthy subjects with thyroid hormones has been reported to lead to increased (34 40) or to unchanged (7) values for the glucose consumption Likewise some authors have found oxygen consumption to be increased (34) whereas others have found no significant change in CO_2 production (7) Several investigators have reported an increased level of glucose 6-phosphate dehydrogenase (18 35 44) and a decreased level of carbonic anhydrase (11 46) in erythrocytes from hyperthyroid patients Disturbances of the electrolyte balance have also been observed Potassium uptake (2) and sodium efflux (39) have been found to be decreased in patients with hyperthyroidism and several authors (5 14 26 39 47) have reported an increase in the erythrocyte sodium concentration

During the recent few years microcalorimetry has been used in investigations of metabolic activities in human cells during normal (3 6 22 23 24 30 31 32 33) as well as pathological conditions (6 21 22 25 30) Levin (21) has reported results of flow calorimetric measurements of heat production in leucocytes thrombocytes and plasma from hyper and hypothyroid patients The heat production in leucocytes was found to be higher in the hyperthyroid group and lower in the hypothyroid group when compared with a group of normal subjects The heat production in thrombocytes and plasma of the same group of patients was found to be normal

In the present study heat production has been measured for erythrocytes of hyperthyroid patients both before treatment and when the patients had become clinically euthyroid For several of the patients measurements were also made at intervals of 4–6 weeks during the period of treatment At each control symptoms were recorded a physical examination was performed and blood was collected for laboratory examinations

MATERIAL

Seventeen patients with clinically definite hyperthyroidism were studied Patients 4 and 6 were taking digoxin because of previously diagnosed arteriosclerotic heart disease (ASHD) with heart failure Neither of these two patients had symptoms or signs of heart failure at the time of the investigation Patient 8 has for several years been treated for asthma with theophylline preparations and sympathomimetics Patient 9 had been thyroidectomized because of thyrotoxicosis several years prior to the present study Patient 15 had been treated intermittently over several years for rheumatoid arthritis but was free from

Table I *Clinical and laboratory data on 17 patients with clinically definite hyperthyroidism*
ASHD=arteriosclerotic heart disease RA=rheumatoid arthritis

Pat no	Sex	Age (y)	T_3U (%)	PBI (µg/l)	T_4I (µg/l)	^{131}I uptake	Heat effect (mW/l) Buffer	Plasma	Glucose consumption (mmol/l h) Buffer	Plasma	2 3 DPG (mmol/l RBC)	Sympt and clinical signs[a]
1	♀	29	206	180	–	High	140 4	–	2 77	–	6 16	27
			80	13	–	–	109 9	–	2 61	–	5 02	6
			100	69	–	–	117 8	–	3 20	–	5 35	20
			114	69	–	–	106 1	–	2 32	–	5 69	13
			102	62	–	–	127 3	–	4 28	–	5 04	20
			105	74	–	–	104 8	–	2 62	–	5 19	11
			110	75	–	–	94 2	–	3 57	–	5 47	7
2	♀	19	150	115	–	–	139 5	–	3 23	–	4 91	21
			87	20	–	–	103 9	–	3 03	–	5 12	6
			114	75	–	–	92 3	–	2 37	–	4 98	4
3	♀	62	143	115	–	–	112 6	–	3 01	–	5 19	11
			83	85	–	–	100 6	–	–	–	5 12	6
			121	106	–	–	100 5	–	1 35	–	5 06	0
4	♂	68	140	105	–	High	111 4	–	3 18	–	4 83	21
			65	26	–	–	83 3	–	2 50	–	4 91	3
			79	65	–	–	92 5	–	1 90	–	4 85	0
5	♀	24	168	120	–	–	111 3	–	2 76	–	5 55	[illegible]
			108	47	–	–	94 2	–	–	–	4 50	5
			76	32	–	–	101 4	–	1 28	–	4 21	[illegible]
			96	40	–	–	82 4	–	1 23	–	4 67	5
			98	49	–	–	94 3	–	–	–	4 98	3
			–	–	–	–	98 5	–	3 11	–	4 84	3
6	♀	67	197	168	–	High	99 6	–	2.17	–	4 82	20
			89	130	–	–	95 7	–	1 48	–	5 06	8
			104	150	–	–	81 8	–	–	–	4 35	3
7	♀	56	191	152	89	High	114 7	110 5	2 79	1 14	–	22
			119	–	49	–	91 7	81 1	1 08	1 38	5 05	7
8	♀	16	226	190	–	–	93 7	91 9	3 54	2 04	5 38	33
			103	–	70	–	102 4	98 7	1 89	1 63	5 33	13
			89	–	59	–	94 2	70 5	2 51	2 43	5 15	13
	♀	35	156	108	95	–	102 1	111 5	2 69	1 92	5 65	21
)			128	–	120	–	89 9	78 9	1 28	1 55	5 34	7
1	♂	45	190	130	95	–	151 3	129 3	3 21	1 79	5 90	33
			128	–	91	–	126 0	92 3	2 74	2 05	5 84	4
11[b]	♂	51	123	86	74	High	89 8	88 7	1 36	–	4 79	23
			93	–	29	–	90 1	77 3	1 65	2 41	5 09	7
			80	–	47	–	108 8	77 3	2 43	1 25	4 88	0
12	♀	73	195	188	–	Normal	87 0	95 2	2 44	1 90	5 20	20
13	♀	55	130	109	–	High	117 5	111 9	2 28	1 71	4 73	29
14	♀	32	137	166	108	High	128 6	102 7	2 42	1 61	4 73	29
			93	–	85	–	121 7	79 0	4 78	2 77	4 89	10
			83	–	78	–	105 3	112 9	–	2 35	4 60	0
15	♀	36	112	160	117	High	91 1	78 5	2 76	1 19	5 50	23
			112	–	103	–	–	90 0	2 68	1 21	4 80	4
16	♀	34	191	168	–	–	88 4	96 9	1 65	1 05	5 88	23
			84	65	–	–	78 8	73 9	1 95	1 81	5 14	1
17	♀	59	139	150	121	High	104 5	–	–	–	6 08	31
			186	200	–	–	126 0	–	4 42	–	5 46	25
			85	–	72	–	80 6	–	1 84	–	–	3

[a] Score according to Crooks et al (8)
[b] The last measurement was made after triiodothyronine had been discontinued

herapy	Other d seases and or treatment
ropyl h ourac l othyrox ne	
mazole levothyrox ne	
arb mazole	
I	ASHD d gox n
arb mazole	
	ASHD d gox n
I	
levothyrox ne	Asthma theophyll ne sympathom metics
hiou ac l levo hyrox ne	Thyro dectomy several years p ev ously
ol propyl hiou ac l	
I tri odo hyron ne	
I	
levothyrox ne	Contracept ve estrogen
levothyrox ne	RA contracept ve estrogen
	Cont a eptive estrogen
I	

symptoms and was not rece v ng any drugs at the t me of the investigat on Patients 14 15 and 16 were tak ng contraceptive estrogens After the d agnos s of hyperthyro dism had been conf rmed 6 of the pa ents were trea ed with I 6 rece ved carb mazole 3 propylth ourac l and one was thyro dectom zed Several of them rece ved thyrox ne and one rece ved tri odothyron ne (Table I)

A group of 6 patients with cl nically suspected hyper thyro d sm was also stud ed (Table II) Two of them had been previously thyro dectomized because of thyrotox cos s (patient 20 several years prior to the present study and patient 21 s x months before the calorimetric observat ons were started) Patient 2? had p ev ously been treated with carb mazole (because of hyperthy ro d sm) This treatment was d scontinued two years before the start of the present invest gation Patient 19 had been treated for one year with d gox n and alprenolol because of atrial f brillation After laboratory conf rma tion of the suspected cl nical d agnos s four patients (nos 19–22) were treated with I patient 18 rece ved propylthiourac l and patient 23 who was thyro d ectomized rece ved thyrox ne

METHODS

Prepa at on of samples

Venous blood was collected nto 10 ml Vacutainer tubes containing 143 USP units of sod um heparin The prepara tion was started with n 10 m n after collect on of blood Samples of erythrocytes were prepared by a centrifuga t on method (method c (30))

The purif ed cells were suspended n autologous plasma wh ch had been made cell free by centrifugat on at 3 000 *g* for 15 min or n glucose phosphate buffer (108 mmol NaCl 3 9 mmol KCl 5 mmol $MgCl_2$ 4 mmol glucose and 20 mmol Na HPO per l tre respect vely the pH was adjusted to 7 40 with phosphoric ac d) The hematocrit varied between 33 7 and 45 5 (mean 40 6) The range of concentration of leucocytes n the suspens on was 0–2 0×10^6/l (mean 0 4) The thrombocytes n the erythrocyte suspens ons we e not counted but from previous experience with the same p eparation method (30) t may be assumed that the average concentrat on of thrombocytes was less than 900×10^6/l The samples of erythrocytes were sto ed at 4 C unt l the calorimetric measure ments were made 2 5–3 hours after the blood was drawn (3)

Calor me c measu emen s

Two nearly dentical twin m crocalorimeters of the heat conduction type were used Samples we e enclosed n teflon-coated stainless steel ampoules volume 1 ml and the measurements were made under stat c conditions The nstrument and ts operation were *briefly described* n the f rst report of this series (30) a more detailed de scription s given elsewhere (45) (cf 42) All calorimetric measurements were performed at 37°C and the resul s re fer to the standard conditions defined earlier (31)

In many cases samples were prepared both with plasma and wi h buffer In these cases two calorimeters were used and the measu ements were performed s mul aneously

Table II *Clinical and laboratory data on 6 patients with clinically suspected hyperthyroidism*
AF=atrial fibrillation

Pat no	Sex	Age (y)	T_3U (%)	PBI (μg/l)	T_4J (μg/l)	^{131}I uptake	Heat effect in buffer (mW/l)	Glucose consumption in buffer (mmol/l h)	2 3 DPG (mmol/l RBC)	Sympt and cl signs[a]
18	♀	26	161	123	–	High	109 2	2 26	5 29	15
			91	50	–	–	101 4	2 34	5 01	5
			104	42	–	–	95 1	1 99	5 21	0
			101	57	–	–	96 4	2 35	5 06	2
			117	53	–	–	86 4	1 22	5 64	0
19	♀	73	144	145	–	Normal	112 7	2 56	4 13	12
			102	145	–	–	107 8	2 32	4 40	1
			106	133	–	–	94 2	1 58	4 64	8
20	♀	38	123	82	–	High	94 5	2 06	5 24	16
			99	43	–	–	92 3	–	5 19	3
21	♀	37	134	110	–	High	94 5	1 89	6 32	12
			120	133	–	–	89 0	1 19	6 49	3
22	♀	66	185	120	89	High	85 9	0 87	6 16	14
			81	–	16	–	94 2	1 03	5 70	6
23	♀	20	162	160	111	–	110 3	2 59	5 81	13
			79	–	117	–	87 9	2 39	5 41	14

[a] Score according to Crooks et al (8)

Other determinations
Hematocrit values were determined using a microhematocrit centrifuge Leucocyte counts on the erythrocyte suspensions were made in a Burker chamber Standard bicarbonate was determined by a standard method PBI was determined with an autoanalyzer (normal values 45–73 μg blood) T_4J was measured by competitive protein binding The method of Seligson and Seligson (38) was used slightly modified (normal values 35–70 μg/l) T_3 uptake was performed according to Hansen (15) (normal values 81–119%) ^{131}I uptake was ...ned after 2 h and after 24 h following oral administration of 15 μCi I (19) Concentrations of 2 3 ...osphoglycerate (2 3 DPG) were measured in duplicate by the enzymatic method of Eriksson and de Verdier (12)

Immediately before and after each calorimetric experiment the glucose concentration in the erythrocyte suspension was measured in triplicate by the God Perid method (Boehringer Mannheim) (37) and the pH was determined using a capillary electrode Radiometer type G 297/G7 The pH measurement immediately after the calorimetric experiment was made on samples taken from the bottom of the calorimetric ampoule where the cells had sedimented

The clinical state of the patients was evaluated by the "clinical diagnostic index (CDI) according to Crooks et al (8) An index from +11 to +19 indicates doubtful toxicity whereas ≥ +20 indicates toxicity

Calculation of results
Heat effect values refer to the time 1 h after the start of the calorimetric experiments and were corrected to pH 7 40 in order to obtain standard values (P^0) as defined elsewhere (31) P values were expressed in mW/l of packed cells (mW/l) Small corrections were applied to for the heat production of leucocytes and thrombocytes present in the suspensions (3) Values determined for the glucose consumption were corrected to pH 7 40 using the correlations found by Garby and de Verdier (13) and are reported as mmol/l of packed cells per h (mmol/l h) Correlation lines were calculated by the method of least squares t test was used for the statistical analysis of the results Uncertainty limits are ±S D

RESULTS

The recorded heat effect values for plasma suspensions remained constant during the experiments For about one third of the samples suspended in buffer the heat effect curves showed a slight declination as described earlier (32)

Patients with Clinically Definite Hyperthyroidism
Patients 12 and 13 were not available for control experiments during the euthyroid state For patient 15 the calorimetric control experiment failed for the measurement with buffer suspension

Measurements in buffer suspension
Heat effects For 17 hyperthyroid patients the mean P^0 value before treatment was 111±20 mW/l (range 87–151) For 14 patients of the same group the mean P^0 value obtained for the euthyroid state was 95±12

Therapy	Other diseases and/or treatment
Propylthiouracil	
^{131}I	AF alprenolol digoxin
^{131}I	Thyroidectomy several years previously
^{131}I	Thyroidectomy 6 months previously
^{131}I	Previously treated with carbimazole
Thyroidectomy levothyroxine	

mW/l (range 79–126) Comparison of P^0 values for the individual cases in their hyperthyroid and euthyroid states showed a highly significant difference ($p<0.001$) (Fig 1)

The initial mean P^0 value was significantly higher ($p<0.001$) than the corresponding value for normal subjects 86±8 mW/l The latter value was derived from our previous results (30) by correction to pH 7 40 Also the final P^0 value in euthyroid state was significantly higher ($p<0.05$) than the normal value The correlation for the individual cases between P^0 values and CDI was highly significant ($r=0.51$ $p<0.001$) The corresponding correlation between P^0 values and the values for the T_3 uptake test was also significant ($r=0.36$ $p<0.05$) whereas the correlation between values for P^0 and PBI before treatment was non significant ($p>0.05$) (Patients 14 15 and 16 were excluded on the grounds that estrogens are known to increase values for PBI and to decrease the T_3-uptake values (16))

Glucose consumption The mean value for glucose consumption before treatment was 2 7±0 7 mmol/l h For the euthyroid state the corresponding value was 2 4±0 9 mmol/l h Both values showed a non-significant difference ($p>0.05$) when compared with the value estimated for normal subjects 1 9±1 1 mmol/l h The estimate was made using the normal value for plasma suspensions 1 3±0 8 mW/l corrected for the difference between plasma and buffer suspensions (32) Comparison for the individual cases of the values for the glucose consumption before and after the treatment showed no significant difference ($p>0.05$) The values for glucose consumption showed a significant correlation with corresponding P^0 values ($r=0.57$ $p<0.001$)

Measurements in plasma suspensions

Heat effects For 10 hyperthyroid patients the mean P^0 value before treatment was 102±15 mW/l (range 78 5–129 3) For 8 patients of the same group the mean P^0 value for the euthyroid state was 85±14 mW/l (range 71–113) Comparison between results for the individual cases showed a significant difference between the hyperthyroid and the euthyroid state ($p<0.05$) (Fig 2)

The initial mean P^0 value was significantly higher ($p<0.001$) than the corresponding value for normal subjects (32) 78±5 mW/l No significant difference ($p<0.05$) was found between the mean P^0 value for the euthyroid state and the normal value

Correlations between both the individual P^0 val

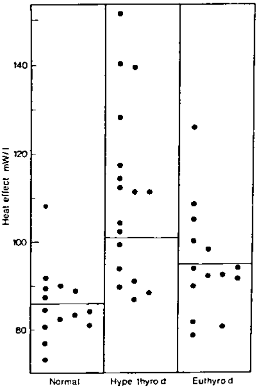


Fig 1 Heat effect values (P^0) for erythrocytes in buffer suspensions Horizontal lines indicate mean values

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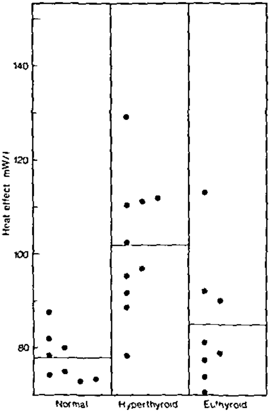


Fig 2 Heat effect values (P^o) for erythrocytes in plasma suspensions Horizontal lines indicate mean values

ues and the corresponding CDI (r=0 55 p<0 01) and between P^o values and the values for the T_3-uptake test (r=0 52 p<0 05) were significant Correlation between values for P^o and PBI before treatment was non significant (p>0 05) Cases 14 15 and 16 were excluded from the comparison

Glucose consumption The mean value for glucose consumption before treatment was 1 6±0 4 and 1 8±0 5 mmol/l h for the euthyroid state There is no significant difference between these values and the mean value previously obtained for normal subjects (32) 1 3±0 8 mmol/l h (p>0 05)

Comparison of the values for glucose consumption for the individual cases before and after the treatment showed no significant difference (p> 0 05)

No significant correlation was found between values for P^o and glucose consumption (p>0 05)

T^3 uptake With the exception of case 15 increased values were found for all patients before treatment Final values in euthyroid state were above normal for 3 patients (nos 3 9 and 10) and at the lower borderline for patients 4 and 11 (Table I) The correlation with CDI was highly significant (r=0 71 p<0 001)

PBI and T_4J test For all patients investigated PBI and T_4J values were found to be above normal before treatment In the euthyroid state PBI was above the normal limit for 4 patients out of 7 while T_4J was still elevated in 5 cases out of 8 (Table I) The correlation between individual values for PBI and CDI before treatment was tested (excluding cases 14 15 and 16 who were taking contraceptive estrogens) and found to be non significant (p>0 05)

2,3 DPG concentration For 13 patients the mean 2 3 DPG value was 5 33±0 38 mmol/l RBC before treatment and 5 02±0 38 in the euthyroid state Comparing results of individual observations obtained in the hyperthyroid and the euthyroid state revealed a significant difference (p<0 01)

Patients with Clinically Doubtful Hyperthyroidism

All experiments were performed with buffer suspensions Results are summarized in Table II

Heat effects The mean P^o value before treatment was 101±11 mW/l (range 86–113) For the same group of patients the P^o value obtained for the euthyroid state was 92±3 mW/l (range 88–96) Comparison between the results for the individual cases did not show any significant change in P^o during the treatment (p<0 05) The initial mean P^o value was significantly higher (p<0 01) than the corresponding value for normal subjects 86±8 mW/l

Correlations for the individual cases of P^o values with CDI T_3 uptake test and PBI were non-significant (p>0 05)

Glucose consumption The mean value for glucose consumption during the calorimetric experiment was 2 0±0 6 mmol/l h before treatment and 1 5±0 5 mmol/l h for the euthyroid state Both values showed a non significant difference (p>0 05) when compared with the mean value previously estimated for normal subjects 1 9±1 1 mmol/l h

Comparison between results for individual cases obtained in hyperthyroid and euthyroid state did not show any difference (p>0 05) A significant correlation was found between the values for glucose consumption and the P^o values (p<0 01)

T_3-uptake test Increased values were found for all patients before the treatment For patient 21 the final T_3 value was at the upper limit and for patient 23 at the lower limit in all other cases the final T_3

value was within the normal range The correlation with CDI was highly significant ($r=0.80$ $p<0.001$)

PBI T_4J For all patients investigated values for PBI and T_4J were above normal before treatment In the euthyroid state PBI was still elevated in 2 patients out of 4 and the value for T_4J was increased in one of the two cases No correlation was found between initial PBI values and clinical condition ($p>0.05$)

2 3 DPG concentration For 6 patients the mean 2 3 DPG concentration was 5 49±0 80 mmol/l RBC before treatment and 5 52±0 61 mmol/l in the euthyroid state

DISCUSSION

Heat effect values for erythrocytes from patients with clinically definite hyperthyroidism were found to be significantly above normal levels ($p<0.001$) before treatment (Figs 1 and 2) There was a good correlation between P^0 values and the CDI The increased P^0 values do not seem to be due to an abnormally large population of young erythrocytes (which are considered to have a high metabolic activity) as the reticulocyte count and the erythrocyte survival time have been found to be normal (4)

In previous studies on normal subjects (32) we found higher P^0 values for erythrocytes suspended in phosphate buffer than for those suspended in plasma The same effect is noted here for cells from hyperthyroid patients and it is believed to be due to the influence of phosphate on the erythrocyte glycolysis (29 36)

For erythrocytes suspended in buffer the mean P^0 value in the euthyroid state was significantly higher than in normal controls

In order to investigate the possible influence of digoxin on the heat production in erythrocytes a few experiments were made where cells from healthy donors had been incubated (1 0 μg/l 0 5 h) with digoxin No effect on P^0 values was found

The significant decrease in 2 3 DPG during treatment is possibly related to the slight increase in Hb concentration from 135 to 141 g/l which was noted during the treatment An inverse correlation has been reported previously between 2 3 DPG and Hb concentration in healthy persons (9 17) However a direct effect of the thyroid hormones as previously reported (28 41) cannot be ruled out

Some of the patients have shown atypical patterns The increased values found at the 3rd and 5th measurements for patient 1 (Table I) corresponded to a worsening of the clinical condition which was of such a degree that the dose of propylthiouracil had to be increased The poor correlation between clinical condition and T_3 uptake as well as PBI tests might be due to high triiodothyronine concentration and normal serum thyroxine level

Several patients showed during treatment increases in P^0 values that could not be related to their CDI (Table I) It cannot be excluded that for some patients P^0 values have been influenced by the administration of thyroid hormones For patient 11 however no decrease in P^0 value was noted when a calorimetric measurement was made 7 months after ending treatment with triiodothyronine

As noted earlier the mechanism of action of thyroid hormones is not known Tata and Widnell (43) have proposed that the peripheral action of thyroid hormones is mediated through their binding to the cell nuclei Obviously this proposal does not apply to the action of these hormones in the non nucleated erythrocytes

Ismail Beigi and Edelman (20) have shown that thyroid hormones stimulate NaK ATPase activity of nucleated cells It is conceivable that a similar stimulation is present also in erythrocytes resulting in an increased heat production Edelman (10) has suggested that the simulation of the NaK ATPase activity is due to an increased membrane permeability for Na^+ and K^+ which would lead to an increased intracellular Na^+ concentration The same author however excluded this possibility after finding a decreased Na^+ concentration in nucleated cells incubated with thyroid hormones Other investigators (5 14 26 39 47) have found an increased Na^+ concentration in the erythrocytes of hyperthyroid patients It therefore seems possible that in these non nucleated cells the primary effect of thyroid hormones is at the cell membrane level whereas in nucleated cells the mechanism of action leading to stimulation of NaK ATPase activity is a different one

It cannot be ruled out that the elevated P^0 values in the hyperthyroid state are due to an increased activity of the pentose phosphate pathway as indicated by the high levels of glucose-6-phosphate dehydrogenase (18 35 44) and increased oxygen consumption (1 34) It can be estimated that for a complete conversion of glucose to CO_2 and H_2O the enthalpy change is about 25 times more exothermic than for the glycolysis process where only lactate

is formed (30 48) This could explain the increased P^0 value found for the hyperthyroid state despite no significantly increased glucose consumption

The basal metabolic rate method has largely been abandoned for the evaluation of the effect of thyroid hormones and it has not yet been replaced by any other method The present results show that calorimetric measurements on erythrocytes will give clinically useful information concerning the peripheral effect of thyroid hormones particularly during changes following treatment of hyper thyroidism

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Serum Vitamin B_{12} Levels in the Aged

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ABSTRACT In an attempt to throw light on the question of age related variations in the normal blood content of cobalamin and on the frequency of deficiencies of antimegaloblastic nutriments in the elderly 273 geriatric patients have been investigated Low serum vitamin B_{12} values were found in one third of these patients, due to latent pernicious anaemia in five and malabsorption in seven cases, and probably caused by nutritional deficiency of folate or cobalamin in 78 cases In that part of the series with apparently normal vitamin B_{12} levels, the mean value (379±14 pg/ml) was lower than the mean (465±20 pg/ml) for a younger control group However, this cannot be taken as a sign of a physiological lowering of the cobalamin values with age as nutritional deficiencies could not be ruled out in this part of the series It is concluded that serum vitamin B_{12} assays should be performed rather liberally in the aged Patients with nutritional deficiency of cobalamin or folate should be treated even if frank megaloblastic anaemia is not present

Measurements of serum vitamin B_{12} concentration in the general population have shown that the level remains relatively constant throughout life (9 10 18 21 24 26) Some investigators (5 6 12 16 17 19 25 27 28 31) have however reported a tendency to decreasing values with advancing age

As pernicious anaemia is predominantly a disease of old age and as nutritional deficiencies are found more frequently at this stage of life it is of importance to evaluate the clinical significance of serum vitamin B_{12} assays in the aged

This study was performed in an attempt to throw light on the question of age related variations in the normal blood content of cobalamin and on the frequency of deficiencies of antimegaloblastic nutriments in a group of elderly people who were in need of geriatric care

MATERIAL AND METHODS

During a period extending over 12 months a total of 349 patients 236 women and 113 men who had been admitted to the Geriatric Centre Århus for various social and medical reasons were studied Some of these patients died shortly after admission others were transferred to another home for the aged and a few failed to attend the investigation for various technical reasons Patients treated with vitamin B_{12} for previously diagnosed pernicious anaemia gastrectomy or other gastrointestinal disorders were primarily excluded as were patients treated with antibiotics at the time of admission In the remaining 273 patients a venipuncture was performed immediately on admission for an assay of vitamin B_{12} by the Lactobacillus leichmannii method (performed by the Research Laboratories of Dumex Ltd Copenhagen) (15)

Patients with normal serum vitamin B_{12} levels i e above 200 pg/ml were not subjected to any further study In patients with low concentrations of vitamin B_{12} an investigation programme was instituted comprising peripheral blood studies (WBC MCV platelets) serum iron total iron binding capacity marrow examination hydrochloric acid in gastric juice after histamine stimulation Schilling test I D-xylose test formiminoglutamic acid (FIGLU) excretion in 5 hour urine samples after histidine load and daily excretion of fat in the stools Finally barium meal studies of the total gastrointestinal tract were performed

The diagnostic criteria for pernicious anaemia were (*a*) low serum vitamin B_{12} concentration (*b*) megaloblastic maturation disturbances in the bone marrow either in the myeloid or erythroid series or in both (2) (*c*) achlorhydria (*d*) pathological Schilling test (3) and (*e*) absence of anatomical defects in the gastrointestinal tract on X ray Only patients fulfilling all five criteria were regarded as suffering from genuine pernicious anaemia and treated with injections of vitamin B_{12} at regular intervals (4) All

Table I. *Serum vitamin B_{12} concentrations related to various haematological and malabsorption studies in 89 geriatric patients*

	Serum vitamin B_{12} (pg/ml)			Total no. of pats
	<100	100–150	150–200	
No. of patients	9	30	50	89
MCV>100 fl			3	72
Leucocytes<2 000/µl	1		2	73
Platelets<150 000/µl				73
Sideropenia	4	6	13	72
Megaloblastic marrow	4	5	9	71
Achlorhydria	4	11	15	69
Schilling test<10%	4	4	7	67
D-xylose test<4.2 g/24 h	2	6	11	67
FIGLU>20 µmol/h	2	9	26	68
Faecal fat>4 g/24 h	7	17	37	68

other patients with low serum vitamin B_{12} levels were kept under observation without any substitution therapy for 6 months, after which period their serum vitamin B_{12} levels were again assayed.

RESULTS

Vitamin B_{12} values above 200 pg/ml were found in the serum of 184 patients. Based on the levels of serum vitamin B_{12}, the remaining 89 patients were divided into three groups (Table I).

In the first group (serum vitamin B_{12}<100 pg/ml) of nine patients, four had non-anaemic genuine pernicious anaemia. Increased urinary excretion of FIGLU suggested folate deficiency in two, and in three patients increased excretion of faecal fat indicated some kind of malabsorption.

The second group (serum vitamin B_{12} 100–150 pg/ml) consisted of 30 patients, of whom only one suffered from pernicious anaemia. Folate deficiency was indicated by the FIGLU test in nine patients.

Table II. *Barium meal studies in 76 geriatric patients with hypovitaminosis B_{12}*

X-ray findings	Bone marrow findings: Normoblastic	Megaloblastic
Normal gastrointestinal tract	30	5
Dilated hypotonic small intestine	15	12
Duodenal diverticulum	7	0
Hiatus hernia of oesophagus	4	0
Neoplasia of gastrointestinal tract	2	1

Of these, four had megaloblastic anaemia and malabsorption of fat and D-xylose was observed in five including one with malabsorption of vitamin B_{12} as well. In another 12 patients there was an excess of faecal fat excretion, including two in whom the Schilling test gave a pathologically low value. One patient had decreased absorption of vitamin B_{12} as assessed by the Schilling test, but did not fulfil the criteria for pernicious anaemia. In seven patients low serum vitamin B_{12} levels were isolated findings.

In the third group (serum vitamin B_{12} 150–200 pg/ml) of 50 patients, no cases of pernicious anaemia were disclosed. Folate deficiency as indicated by the FIGLU test was seen in 26 patients. Of these five also had malabsorption of vitamin B_{12}, while 13 had malabsorption of fat and/or D-xylose. In two patients, only the absorption of vitamin B_{12} was depressed. In another 22 patients, faecal fat was increased. Seven patients with megaloblastic transformation in the marrow aspirate had a positive FIGLU test.

All patients with sideropenia (low serum iron and high total iron binding capacity) had normoblastic bone marrow.

The patients subjected to barium meal studies were divided into two groups, one with normoblastic and the other with megaloblastic bone marrow. Table II shows that most of the patients with a normal X-ray appearance of the gastrointestinal tract had normoblastic marrow. However, a dilated hypotonic small intestine was a fairly common finding, and many of these patients had megaloblastic bone marrow. An unsuspected neoplastic process in the gastrointestinal tract was revealed in three cases.

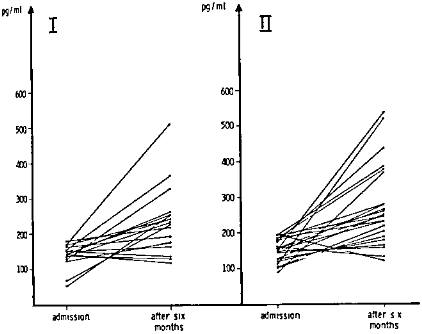


Fig 1 Serum vitamin B_{12} concentrations in 17 geriatric patients with normal Schilling and FIGLU tests (*I*) and in 23 patients with normal Schilling test but pathological FIGLU test (*II*)

Six months after admission the serum vitamin B_{12} concentration was determined again in 40 patients without pernicious anaemia (Fig 1) Among 17 patients with normal Schilling and FIGLU tests on admission the serum vitamin B_{12} level had increased significantly in all but three ($p<0.005$) In 23 patients who had originally showed a normal Schilling test but a pathological FIGLU test the serum vitamin B_{12} had also increased significantly ($p<0.001$)

After exclusion of patients with pernicious anaemia ($n=5$) malabsorption of vitamin B_{12} ($n=7$) folate deficiency ($n=37$) and patients with initial low vitamin B_{12} values increasing to normal ($n=14$) a total of 202 geriatric patients was left As we had reason to believe that low serum vitamin B_{12} levels observed in another 26 patients should be attributed to nutritional deficiency these were also excluded leaving a number of 184 elderly individuals with apparently normal serum vitamin B_{12} concentrations Table III shows that the mean value for vitamin B_{12} was 379±14 pg/ml lower in men than in women The difference is significant ($p<0.001$) A comparison of this mean value with that (465±20 pg/ml) in a reference material (14) revealed a significant difference ($p<0.001$)

The frequency distribution diagram of the serum vitamin B_{12} concentrations (Fig 2) was strongly suggestive of a logarithmic distribution and this was confirmed by the probit diagram (Fig 3)

The 2s range (0 95 level) for subjects of advanced age may be defined from Fig 3 as 110–910 pg/ml

DISCUSSION

In the series studied consisting of 273 geriatric patients serum vitamin B_{12} values below 200 pg/ml

Table III *Serum vitamin B_{12} levels in 184 geriatric patients*

	No of cases	Age (y)	Serum vitamin B_{12} (pg/ml) Range	Mean ±S E M
Women	123	80±7	202–1 455	405±20
Men	61	79±7	202– 796	337±16
Total	184	80±7	202–1 455	379±14

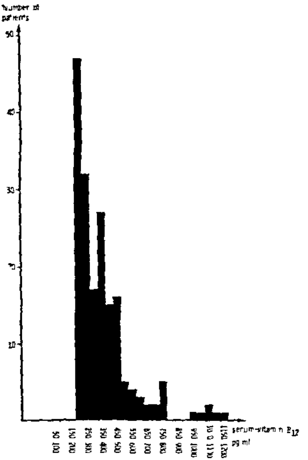


Fig 2 Distribution of 184 geriatric patients related to serum vitamin B_{12}

were present in 89 cases i e approximately in one rd of the series

Genuine pernicious anaemia was found in five patients This is in agreement with a previously observed incidence of approximately 1% in the general population of the same age range (29) It is remarkable that none of these patients were anaemic and that peripheral blood studies did not reveal any signs indicative of pernicious anaemia Nevertheless their bone marrow showed megaloblastic transformation This suggests that latent pernicious anaemia (1 13 22) is not an uncommon phenomenon in old age In these latent cases the serum vitamin B_{12} concentrations were within the same low range (<100 pg/ml) as is seen in manifest pernicious anaemia As a myelopathy may develop before anaemia occurs (3) it is reasonable to perform cobalamin assays liberally in patients of advanced age

Among the remaining 84 subjects who had low serum vitamin B_{12} concentrations without manifest pernicious anaemia 37 showed a pathological FIGLU excretion test This test was performed in order to segregate the cases in whom a low serum vitamin B_{12} concentration might be due to folate deficiency The FIGLU test will be positive in most cases of folate deficiency which is severe enough to affect cellular metabolism However the test may also be positive in cases of severe vitamin B_{12} deficiency Knowles and Prankerd (20) found pathological FIGLU tests in patients with severe pernicious anaemia whereas the test was normal in cases with Hb levels above 8 8 g/100 ml As our patients had normal Hb levels it is likely that a large proportion of those with abnormal FIGLU test actually suffered from folate deficiency and that the subnormal vitamin B_{12} concentrations were secondary to this deficiency

As nutritional folate deficiency is fairly common in the elderly (26 30) it is reasonable to assume that dietary insufficiency was responsible in many of these cases This assumption is supported by the fact that the serum vitamin B_{12} levels increased during their stay in the Geriatric Centre when they were offered a better dietary supply

There were 47 subjects with serum vitamin B_{12} levels below 200 pg/ml but normal FIGLU tests However these values can only be regarded as physiological for this age group if other causes such as intestinal malabsorption and dietary insufficiency can be unquestionably ruled out

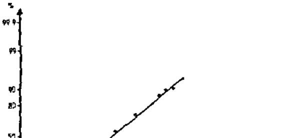


Fig 3 Cumulative frequency of serum vitamin B_{12} in 184 geriatric patients

Gastrointestinal dysfunction as indicated by increased faecal fat depressed Schilling test and D-xylose test was found with a relatively high frequency Flocculation and dilatation of the small intestine were outstanding features In many cases with X ray abnormalities a megaloblastic bone marrow was encountered Impaired absorption of vitamin B_1 resulting from this malabsorption syndrome had probably contributed to low serum vitamin B_{12} levels in seven cases

Even today some elderly people in our community feed badly either for socio economic reasons or because of physical or mental disability It must be admitted that owing to the relatively small folate depots of the body an inadequate diet may more readily give rise to a deficiency of folate than of cobalamin That a dietary cobalamin deficiency may nevertheless develop is known from the occurrence of megaloblastic anaemia in vegans (32)

In the series presented here there was a group of 40 individuals in whom pernicious anaemia folate deficiency and intestinal malabsorption could not be incriminated for the low serum vitamin B_{12} values and in whom an insufficient dietary supply of vitamin B_{12} must therefore be seriously considered In three patients in whom megaloblastic transformation was revealed in the bone marrow it is not acceptable to regard the low cobalamin values as merely a normal physiological state of old age The same applies to the 17 non folate-deficient individuals in whom a rise in serum vitamin B_{12} occurred after they had been placed on a normal hospital diet Although a single injection of vitamin B_{12} was given in conjunction with the Schilling test it is well documented that after a few weeks delay the serum vitamin B_{12} concentration returns to its original level in cobalamin-deficient patients (23) Thus this observation is in favour of the assumption that the low serum vitamin B_{12} levels were due to a nutritional deficiency

After the exclusion of these 40 patients 184 individuals remained with apparently normal serum levels of vitamin B_{12} Although the mean value of serum cobalamin in these patients is still significantly lower than the mean for the control group of younger subjects the difference is so small and other causes of low serum vitamin B_1 levels so frequent in the aged that it seems rather unlikely that the blood content of cobalamin should normally decrease with advancing age As pointed out by Cape and Shinton (5) the divergent results obtained by various authors may be due to differences in the methods of selecting individuals for study as some reports are based solely on sera from elderly persons admitted to an institution for some time

Further it must be concluded that routine investigations for folate and cobalamin deficiency in geriatric patients may be worthwhile as a hitherto undiscovered folate and/or cobalamin deficiency may be present in many cases A small number of these patients will have latent pernicious anaemia but the majority are patients in whom a nutritional deficiency has resulted in folate and/or cobalamin deficiency Long standing deficiency of these vitamins may lead to a malabsorption syndrome further aggravating the symptoms (7 8)

Once revealed a deficiency state should be treated even if frank megaloblastic anaemia is not present *Folate deficiency* is appropriately treated with oral folic acid in daily doses of 100–200 μg as such small doses will not interfere with a possibly co-existing cobalamin deficiency (12) *Latent pernicious anaemia* needs adequate initial and maintenance therapy just like the frank variety of the disorder In *nutritional cobalamin deficiency* vitamin B_{12} should be given in doses capable of restoring the normal content of the body In order to prevent neurological and mental manifestations of the deficiency it seems to be of importance to replenish the stores which are approximately 4 mg The fulfilment of this requirement is best obtained by injections of cobalamin either in the form of hydroxycobalamin 8 mg in eight doses or depot cobalamin (Betolvex®) 4 mg in four doses (4)

Whether the treatment should be continued as maintenance therapy in cases of nutritional deficiency depends on the extent to which it is possible to induce the patients to take an adequate diet If it proves difficult for the patients to change their dietary habits which is often the case in the aged maintenance must be given both in folate and cobalamin deficiency

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Iron Therapy in Patients Undergoing Maintenance Hemodialysis

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ABSTRACT Twelve patients in maintenance hemodialysis, receiving long term oral iron therapy, have been treated with i v iron dextran in order to evaluate the effect on the Hb level Both Hb and hematocrit were unchanged before and after the iron dextran infusion ($p>0\ 5$, $p>0\ 7$, respectively) Oral iron therapy is usually sufficient to maintain an adequate iron balance in dialysed patients and should be preferred to parenteral iron in view of the better utilization and absence of side effects The indication for parenteral iron should be limited to patients with impaired gastrointestinal iron absorption

Anemia is a constant and inevitable feature in patients undergoing regular hemodialysis treatment (RDT) (5 12 22 23) Its pathogenesis is complex and it appears to be caused both by inadequate red cell production due to lack of erythropoietin (10 12) and by accelerated red cell loss due to hemolysis and bleeding (5 12 23) The appreciable blood loss in connection with dialysis makes iron supplementation necessary in order to avoid iron deficiency (19 23)

It has been claimed that parenteral iron induces a rise in the Hb level in dialysed patients with replete iron depots (30) and the diverging opinions concerning the efficiency of oral versus parenteral iron therapy (1 6 18 23) prompted the present study with the purpose of assessing whether it is possible to achieve a further rise in Hb by the administration of parenteral iron to hemodialysis patients receiving long term oral iron treatment

MATERIAL AND METHODS

Twelve patients participated in the study (Table I) All had been on RDT for 9–41 months (mean 21) and were on a protein restricted diet containing an average of 0 9 g protein/kg b wt /day together with vitamin supplements Dialysis was performed for 10 hours twice weekly using the Gambro-Lundia® artificial kidney and the degree of uremia was constant during the study Blood sampling was restricted to a minimum blood transfusion avoided and no extraordinary blood loss observed in the investigation period None of the patients had been subjected to gastrointestinal surgery or had symptoms of malassimilation or infection All received oral iron as ferrous fumarate 200 mg (66 mg elemental Fe^{2+}) together with ascorbic acid 250 mg thrice daily during 4–16 months (mean 10) before the study Iron dextran (Imferon®) containing 500 mg elemental Fe^{3+} was administered to each patient in 500 ml 0 9% saline during dialysis

Bone marrow specimens were obtained by iliac crest puncture before the iron dextran infusion and the hemosiderin iron content was assessed after staining with Prussian blue (25) Hb hematocrit mean corpuscular volume (MCV) mean corpuscular Hb concentration (MCHC) corrected reticulocyte count serum iron plasma total iron binding capacity (TIBC) and plasma transferrin saturation were measured before the infusion of iron dextran and at regular intervals up to 40 days thereafter employing the methods described in earlier papers (20 23)

In statistical analysis the Wilcoxon rank sum test was used to evaluate significance of differences

RESULTS

One patient (no 1) had no detectable marrow hemosiderin iron while the others had normal or slightly reduced hemosiderin iron stores (Table I) None of the patients presented with evidence of iron deficiency judged by the serum iron transferrin saturation TIBC MCV and MCHC which were all within the normal range

The iron dextran infusion was followed by a transient moderate rise in the reticulocyte count reaching its maximum 5–10 days after the infusion (Fig 1 and Table II) However the mean Hb and hematocrit values were unchanged after the infu

Table I *Clinical data marrow hemosiderin duration of hemodialysis and of oral iron therapy in patients receiving iron dextran infusion*

Pat no	Sex	Age (y)	Dialysis before iron dextran (mo)	Oral iron treatment before iron dextran (mo)	Marrow hemosiderin before iron dextran (0–4+)	Renal disease
1	♀	50	10	6	0	Chronic glomerulonephritis
2	♀	38	20	16	1+	Chronic pyelonephritis
3	♀	38	35	16	1+	Chronic glomerulonephritis
4	♀	38	9	4	1+	Primary oxalosis
5	♂	32	10	4	2+	Chronic glomerulonephritis
6	♂	54	12	7	2+	Chronic glomerulonephritis
7	♂	32	40	11	1+	Chronic glomerulonephritis
8	♂	52	41	9	2+	Chronic glomerulonephritis
9	♂	23	35	13	2+	Hereditary nephropathy
10	♂	26	15	10	2+	Chronic glomerulonephritis
11	♂	42	19	12	2+	Nephrosclerosis
12	♂	18	11	11	2+	Chronic glomerulonephritis

sion Hb averaged 3 9±0 9 (S D) mmol/l before and 3 9±0 8 mmol/l 40 days after the iron infusion (p>0 5) while the hematocrit averaged 0 19±0 04 both before and after the infusion (p>0 7) (Fig 1 and Table II)

The administration of iron dextran induced a significant rise in serum iron and the transferrin saturation and the plasma iron binding capacity was exceeded in several patients (Fig 2) Serum iron returned to pretreatment levels within 20 days after the iron dextran infusion while a slight permanent increase in the transferrin saturation was observed along with a slight decrease in TIBC (Table II) MCV and MCHC did not change significantly during the study

Analysing the individual response of the patients it appeared that one (no 1) demonstrated a pronounced reticulocytosis following the iron dextran accompanied by a distinct rise in Hb from 2 7 to 4 3 mmol/l and in the hematocrit from 0 14 to 0 19 TIBC fell from 64 4 to 53 4 µmol/l the transferrin saturation was unchanged (from 19 9 to 21 7%) and so was serum iron (from 12 8 to 11 6 µmol/l) while MCV rose from 89 to 95 fl

The iron dextran infusions were generally well tolerated and not accompanied by serious adverse reactions

DISCUSSION

Intravenous iron dextran has been claimed to be effective in raising the Hb level in iron deficient dialysis patients (6 7 14 18 24) as well as in

Table II *Hematological data (mean±S D) before and after iron dextran infusion in 12 patients in maintenance hemodialysis receiving long term oral iron therapy*

	Days after iron dextran						
	0	5	10	20	30	35	40
Hb (mmol/l)	3 9±0 9	3 9±0 8	3 9±0 9	3 9±0 8	3 9±0 7	3 9±0 8	3 9±0 8
Hematocrit	0 19±0 4	0 19±0 4	0 19±0 4	0 19±0 4	0 19±0 4	0 19±0 4	
Corrected reticulocyte count (0/00)	14±6	24±8*	24±15**	20±10**	18±8		
MCV (fl)	98±6			100±4		101±4	
MCHC (mmol/l)	20 6±0 7			20 5±1 3		20 8±1 1	
Serum iron (µmol/l)	18 2±6 8	52 3±6 4*		17 3±6 7	18 1±6 5		
TIBC (µmol/l)	52 4±8 1	51 6±10 0		50 8±8 7	46 1±7 3**		
Transferrin saturation (%)	36 4±15 1	103 4±18 4*		38 5±16 5	42 7±22 2**		

Significant difference from day 0 * p<0 01 * p<0 05

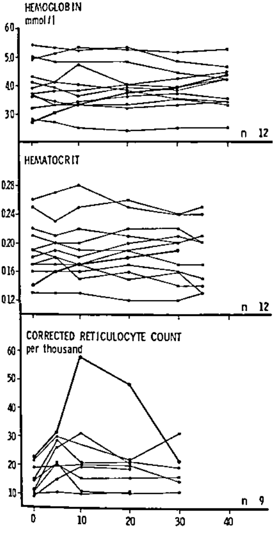


Fig 1 Hb hematocrit and corrected reticulocyte count before and after iron dextran infusion in hemodialysis patients receiving long term oral iron therapy Accentuated line=case 1

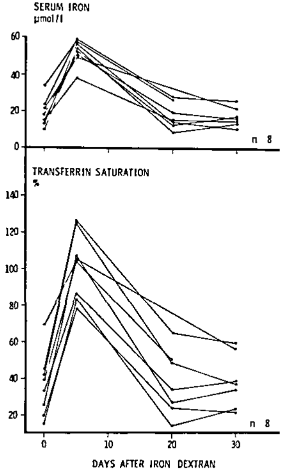


Fig 2 Serum iron and transferrin saturation before and after iron dextran infusion in hemodialysis patients receiving long term oral iron therapy

patients with replete iron depots (30) and is often recommended in doses exceeding the actual iron loss (16 18) However serious side effects have been reported in connection with this treatment (3 21) and long term administration carries the risk of iron overload (8 21 30)

Iron dextran is primarily cleared by the reticuloendothelial system (3 21) and hereafter only 60–70% becomes available for Hb formation in normal persons (21 29) The utilization is even lower in uremic subjects due to impaired release of iron from the iron dextran complex in the reticuloendothelial cell (2)

Several reports indicate that iron absorption is normal in uremic patients and subjected to the regulatory influence of iron balance as in healthy individuals (5 11 23) Oral iron therapy seems effective in the prevention and treatment of iron deficiency in dialysis patients (1 14 23 26 28) and in repleting marrow iron depots with a finely distributed easily utilizable form of iron (1 14 27) while iron dextran causes a granular form of iron depots that are hard to mobilize (14) Oral iron treatment is free from serious side-effects and the risk of iron overload is negligible If necessary iron absorption can be increased by addition of ascorbic acid and administration between meals (13 15)

The present study suggests that oral iron supplementation is generally sufficient to maintain an

adequate iron balance and repleted iron stores in patients on RDT We observed no advantageous effect of parenteral iron on the Hb level in patients receiving oral iron and having replete iron stores However one patient (no 1) with probable iron deficiency and absent hemosiderin iron stores responded to parenteral iron with a distinct rise in Hb the failing effect of oral iron treatment in this case was caused by impaired absorption due to achlorhydria

It is generally accepted that serum iron is an unreliable index of iron stores in dialysis patients (4 8 9 23 24 27, 30) TIBC and transferrin saturation are likewise influenced by other factors than the iron supply (18 23) and are therefore inaccurate indicators of the iron status Iron treatment in dialysis patients having normal TIBC and transferrin saturation (but unassessed iron stores) has been followed by a rise in Hb (6 7 28) suggesting iron deficiency Also it is noticeable that the iron deficient patient in this study had serum iron TIBC and transferrin saturation values within the normal range

Summing up both parenteral and oral iron seem effective in preventing and correcting iron deficiency in hemodialysis patients Oral iron treatment is advocated in view of the absence of side effects the deposition of marrow iron in a readily mobilizable form, the small risk of iron overload and the higher utilization compared with parenteral iron (2 23) The indication for parenteral iron should be limited to patients with gastrointestinal disorders impairing the absorption of orally administered iron

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A New Pattern of Multiple Endocrine Adenomatosis

Chemodectoma Bronchial Carcinoid GH producing Pituitary Adenoma and Hyperplasia of the Parathyroid Glands and Antral and Duodenal Gastrin Cells

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ABSTRACT A female patient was found to have a chemodectoma, a GH producing pituitary tumour and a bronchial carcinoid combined with hyperplasia of the parathyroids and of antral and duodenal gastrin cells This combination of endocrine tumours and hyperplasias does not fit with the two multiple endocrine adenomatosis syndromes recognized at present The case stresses the importance of scanning the patient for other endocrine tumours once one has been diagnosed

The simultaneous occurrence of two or more endocrine tumours in the same patient has long been recognized This syndrome is sometimes referred to as multiple endocrine adenomatosis (MEA) Some combinations of endocrine tumours are more common than others The association of pituitary and parathyroid tumours with islet cell tumours constitutes MEA syndrome type I or Wermer s syndrome (3 33) To this combination is sometimes also added adrenocortical and thyroid tumours and sometimes endocrine lung and intestinal tumours (19) The MEA syndrome type II or Sipple s syndrome consists of the association of thyroid medullary carcinoma with phaeochromocytoma and sometimes also with parathyroid adenomas or hyperplasia (28) Parathyroid tumours or hyperplasia may thus occur in both syndromes In addition there are some reports of MEA syndromes with associated thymic tumours (26)

The case was briefly reported at Lakaresallskapets Riksstamma Stockholm 1975 Acta Soc Med Suec 84 (4) 88 1975

The present report describes a patient with an unusual combination of hyperplasias and tumours of five or possibly six endocrine cell systems This combination does not fit either of the two MEA syndromes

CLINICAL DATA

A I para female patient born in 1929 had in 1962 a duodenal ulcer which was cured by medical treatment only In 1965 she was admitted for investigation of head ache and partial loss of hearing on her right ear Signs of acromegaly were noted upon admission Laboratory findings were hypercalcaemia (S Ca 2 7 mmol/l) hypophosphataemia (0 35 mmol/l) and hypercalcuria (dU Ca 17 5 mmol) Roentgenogram of the abdomen demonstrated a renal calcification the size of a grain of rice X ray examination revealed slight enlargement of the sella turcica. The visual fields were normal Thyroid tests (basal metabolic rate +10% and +14% and protein bound iodine 7 4 μg/100 ml) were normal

Exploration of the neck revealed three parathyroid glands of which two were enlarged and the third was of normal size The two enlarged glands were removed (the left weighed 3 7 g the lower right 0 6 g) and partial resection of the normal sized right gland was carried out Enlargement of the thyroid which also contained multiple nodules led to a bilateral subtotal thyroidectomy The histological diagnosis was hyperplasia of the parathyroid glands while the thyroid lobes were normal except for a slight nodularity The postoperative course was uneventful and S-calcium returned to normal levels The patient was substituted with thyroxin

In 1968 a myoma of the uterus was extirpated At that time the patient showed slight hypertension In 1971 a chest X ray revealed a tumour mass (12 mm in diameter) in the middle lobe of the right lung A partial resection of the affected lobe was performed and the



Fig 1 Fine needle punction from the mediastinal tumour showing a cluster of cells The cells are uniform in size with scanty cytoplasm and rounded nuclei There is a tendency to form small acinar structures Hematoxylin eosin ×1 000



Fig 2 Aspiration biopsy smear from the retromandibula tumour showing elongated cells with ovoid nuclei an abundant cytoplasm The cytoplasm is finely granular Hematoxylin eosin ×1 000

tumour was found to be connected with a small bronchus The histological diagnosis was bronchial adenoma car cinoid type alveolar growth pattern with lymphatic in vasion

In 1972 and 1973 the patient experienced gastric ulcers that healed on medical treatment In 1973 chest X rays revealed a tumour measuring 8×11 cm in the central and posterior part of the thorax No relation to the thymic region could be established Fine needle aspiration biopsy of this tumour revealed a picture compatible with a car cinoid metastasis (Fig 1) A right retromandibular tumour was also detected Angiography of the right common carotid artery demonstrated a tumour 7 cm long ex tending from the carotid bifurcation to the jugular fora men The tumour displaced both carotids and compressed the internal carotid Fine needle biopsy permitted a tenta tive diagnosis of chemodectoma (Fig 2)

Laboratory findings Basal acid output 8 3 mEq/h increased markedly after pentagastrin stimulation (to 64 mEq/h) Fasting B glucose and glucose tolerance were within normal limits as was S insulin S calcitonin S TSH S-diamine oxidase dU VMA dU-catecholamines and dU HIAA were also normal S-GH was not analyzed

At exploration (in 1973) in hypotension anaesthesia the neck tumour was found to adhere to surrounding tissues At the level of the bifurcation the vagal nerve separated into 3 or 4 bundles entered the caudal portion of the tumour The tumour enclosed both carotids The hypo-glossal nerve also entered the tumour but was not di vided into bundles like the vagal nerve The external carotid artery was ligated As the tumour encircled the internal carotid artery it could not be resected en bloc and had to be removed stepwise leaving an inaccessibl part adjacent to the jugular foramen During the dissec tion the bundles of the vagal nerve could not be identi fied Postoperatively the patient never regained con sciousness Angiography revealed embolism and infarc tion of the right cerebral hemisphere The patient die seven days after operation

Relevant autopsy findings Remnants of neck chemo dectoma Small thrombus in the right carotid bifurcation Thromboembolic material in the small vessels of the righ cerebral hemisphere with infarction Limited part necrotic tumour in the sella turcica Tumour metastase in the right lung and the mediastinum Prepyloric scar Nodular hyperplasia of the adrenal cortex Slight acut agonal pancreatitis No parathyroid glands were found

MATERIAL AND METHODS

Surgical specimens and autopsy material (about 3 h po mortem) were fixed in 4% formaldehyde dehydrated i graded ethanol solutions and embedded in paraffir Part of the material was also frozen to the temperatur of liquid nitrogen in a propane propylene mixture freez dried and exposed to formaldehyde gas at 80°C for 1 (5) Control specimens were heated in the absence (formaldehyde Subsequently all specimens were em bedded in paraffin in vacuo sectioned at 5 μ mounte in Entellan (Merck) and examined in a Leitz Orthopla fluorescence microscope equipped with an epi illumina tion system (standard filter setting no 2 peak excitatic at 405 nm) The light source was an HBO 200 mercur lamp



Fig 3 Bronchial tumour at operation in 1965 Regular epithelial cells are arranged in densely packed cords A few acinus like structures are seen Hematoxylin-eosin ×18?



Fig 4 Neck tumour at operation in 1973 The tumour is in this part made up of groups of elongated slightly irregular cells in a stroma rich in capillaries The picture is typical of chemodectoma Most of the tumour had a mesenchymal pattern van Gieson ×18?

Sections from formalin fixed or freeze-dried material were stained with hematoxylin eosin Masson alkaline Congo red (??) cresyl violet at pH 4 0 and pH 5 0 preceded by short acid hydrolysis (18 29) aldehyde fuchsin (15) silver impregnation according to Grimelius (1?) and Hellerstrom and Hellman (13) and the periodic acid alcian blue PAS Orange G stain (1)

Gastrin and GH were demonstrated by an indirect immunohistochemical technique The rabbit anti gastrin serum (no 2609) has been characterized in detail elsewhere (24) The anti-GH serum was raised in rabbits by subcutaneous injection of 0 75 mg human GH in 0 5 ml 0 9% saline mixed with 0 5 ml Freund s complete adjuvant Five injections were given by this route at 14 days intervals The anti gastrin serum and the anti-GH serum were applied as first layers in dilutions 1 10 and 1 5 respectively The second layer consisting of a goat antiserum directed against rabbit IgG labelled with fluorescein isothiocyanate (Miles) was used in dilution 1 ?0 After mounting in buffered glycerine the sections were examined in the fluorescence microscope (Standard filter setting no 3 peak excitation at 490 nm) Controls were those recommended by Goldman (10) and included the application of antigen inactivated antiserum (14 17)

Microspectrofluorometry

Fluorescence spectra were analyzed in a modified Leitz microspectrograph (4) Differentiation between the fluorophores of the two catecholamines noradrenaline and dopamine was performed by stepwise acidification of the tissue sections as described by Bjorklund et al (4)

RESULTS AND COMMENTS

Parathyroid glands The two enlarged parathyroid glands contained no remnants of normal glandular tissue Both these glands showed hardly any fat tissue The normal sized gland also showed only a few fat cells The parenchymal cells were arranged in a nodular pattern Some large areas were acinar All three glands showed a similar cell composition with roughly the same number of chief cells (dark and light) and oxyphil and transitional oxyphil cells The multiglandular arrangement and the absence of remnants of normal glandular tissue outside the connective tissue capsule favour the diagnosis of hyperplasia (chief cell type) The fourth gland could not be found at the operation This gland may have never developed but it is perhaps more likely that the two left glands had fused an interpretation that is supported by the nodularity of the tissue and the failure to find parathyroid glands at autopsy

Thyroid gland The thyroid lobes showed a slightly nodular arrangement of the follicles Silver impregnation according to Grimelius (1?) revealed only a few C cells

Bronchial tumour The primary tumour was situated in the wall of a small bronchus The tumour cells formed small groups often in an alveolar arrangement (Fig 3) and were also found in small vessels in the neighbourhood of the main tumour Some tumour cells were slightly argyrophilic in Grimelius staining but they were all negative in Masson s argentaffin staining No amyloid could be demonstrated

Mediastinal tumour The tumour material was

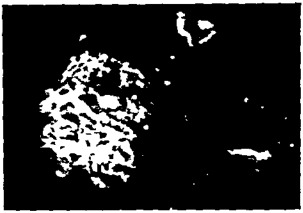


Fig 5 Chemodectoma. Freeze-dried formaldehyde vapor-treated tissue (obtained at surgery) A nest of intensely fluorescent cells is seen together with scattered solitary tumour cells The fluorescence is characteristic of dopamine ×182.

largely necrotic In better preserved areas the tumour cells had an appearance similar to the bronchial tumour but slightly more anaplastic No amyloid could be demonstrated The tumour cells were slightly argyrophil but not argentaffin. No formaldehyde-induced fluorescence was seen. Together these data strongly suggest that the tumours represented metastases from the bronchial tumour The absence of formaldehyde induced fluorescence might be explained by the fact that 3 hours elapsed before the material was frozen. Furthermore these tumours are said to produce 5-HT only rarely

Neck tumour The tumour showed a spindle cell or mesenchymatous growth pattern in most parts but also contained groups of epithelium like cells with moderate nuclear polymorphism The tumour was rich in blood capillaries and was surrounded by a condensation of connective tissue (Fig 4) Most of the tumour cells were argyrophil with the Grimelius technique and stained with cresyl violet at pH 5 0 (but not at pH 4 0) after hydrolysis No amyloid was found The tumour cells were negative in Masson s argentaffin reaction

In freeze-dried formaldehyde treated sections of the chemodectoma tissue a large number of cells emitted an intense greenish fluorescence The cells were usually elongated or spindle shaped and sometimes had long fluorescent processes They occurred either singly or in small groups occasionally they were seen collected together in round nests or glomeruli consisting of an abundance of fluorescent cells and their processes (Fig 5) The cellular fluorescence had maximum excitation a 410 nm and maximum emission at 475 nm Up acidification the excitation maximum shifted 370–380 nm this maximum persisted after longed acidification The spectral properties corded are characteristic of dopamine

The general appearance of the tumour as we as its content of catecholamines are strong eviden in favour of the diagnosis of chemodectoma Su features however are unusual in carotid bod tumours—the most common type of toma. Thus the present tumour contained mine while carotid body tumours have described to contain noradrenaline The close a sociation of the tumour with the vagal nerv suggests that it represents instead a vagal dectoma (8)

Pituitary tumour The material in the sella turcica was partly necrotic In areas w served cells these were mostly orangeophilic Pearse s staining and showed strong GH reactivity This finding together with the patient acromegalic features justifies the diagnosis of G producing acidophilic pituitary adenoma.

Antral and duodenal mucosa chemistry revealed a marked hyperplasia of cells both in antral and in duodenal mucosa. In antral mucosa the gastrin cells were clumped together in small nests in an like fashion (Fig 6) No such nests were in the duodenal mucosa where the most

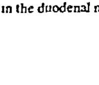


Fig 6 Antral mucosa. Formalin-fixed tissue (taken autopsy) Immunofluorescence reveals a much number of disseminated gastrin cells than normally ×125 Most cells occur together in clusters (inset) ×2

hyperplasia involved the gastrin cells of the Brunner s glands

Pancreas The number of islets appeared normal but some islets were rather large The pancreas showed a slight acute pancreatitis which probably accounts for difficulties in demonstrating the islet cells with special stains In the islets weakly reactive B and A_2 cells were demonstrated but no A_1 cells

Kidneys There were a few small groups of lymphocytes a few casts and some very small calcifications There was no reason to believe that the parathyroid hyperplasia was due to renal changes

Adrenal glands These were slightly nodular No tumour was found

DISCUSSION

Different peptide hormone producing tumours sometimes coexist Two separate patterns of concurrence of these tumours have been recognized multiple endocrine adenomatosis (MEA) syndromes I and II as described in the introduction These have recently been reviewed (6 19 20 32) Occasionally the multiple endocrine adenomatosis syndrome has been described to be associated with bronchial or intestinal carcinoids (9 30 31 34) Mediastinal tumours (thymic carcinoids?) have also been reported to be engaged in an MEA syndrome (?6) Chemodectoma a tumour originating from chemoreceptor tissue may occur together with tumours of the endocrine pancreas (11) bodies of Zuckerkandl (7) thyroid (2) and adrenal medulla (25 27) The basic derangement leading to the multiple endocrine adenomatosis is probably due to genetic factors

In the present case three different endocrine tumours occurred—a GH producing pituitary tumour a bronchial carcinoid and a chemodectoma probably of vagal origin In addition hyperplasia was seen in two endocrine organs or organ systems —the parathyroid glands and the gastrin producing cells in the gastric antrum and in the duodenum The nodular hyperplasia of the adrenal glands may be a secondary phenomenon and not a primary manifestation of the syndrome (32)

The cause of the peptic ulcers might have to do with the hyperplasia of the gastrin producing cells

A common origin of peptide hormone secretory cells has been suggested the neurectoderm with the possible exception of the parathyroid glands (19 20 32) This exception is now debated and Pearse (20) claims to have demonstrated an ectodermal origin of the parathyroids However it has recently been questioned whether the pancreatic islets develop from the neural crest as excision of the latter does not prevent the appearance of islet cells (21) The unitarian view of the origin of endocrine polypeptide producing cells—that all should originate from the neurectoderm—emphasized by the designation neurolophomas by Pearse may therefore not be valid The migration of pancreatic islet cells from the neural crest may however take place so early in the ontogenesis that it cannot be prevented by excision of the neurectoderm (Takor Takor personal communication)

For several reasons present knowledge of the multiplicity of peptide hormone producing tumours seems incomplete For instance it has recently been demonstrated that a substantial proportion of pancreatic endocrine tumours are multihormonal The secretion of more than one hormone from these tumours was not apparent clinically (16) This has been noted with other endocrine tumours as well (23)

The present case further underlines the value of scanning patients for hypersecretion of other peptide hormones once one endocrine tumour has been diagnosed

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Multiple Endocrine Adenomatosis of Mixed Type

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ABSTRACT A case of multiple endocrine adenomatosis (MEA) of mixed type is presented The syndrome observed in a 65 year old female, consisted of multiple neurofibroadenomatosis medullary thyroid carcinoma, multiple adenomata of the parathyroids adrenal cortical adenoma and small cell anaplastic bronchogenic carcinoma Thus, it was composed of type 1 as well as of type 2 MEA On the basis of another seven cases, collected from the literature, the MEA *syndrome of mixed type* is reviewed with special reference to the phylogenetic origin of the cells of the APUD system

The occurrence of several tumours with peptide secreting capacities in the same individual has been designated multiple endocrine adenomatosis (MEA) The clinical distinction of familiar MEA into two different syndromes Wermer s syndrome (MEA_1) and Sipple s syndrome (MEA_2) is in agreement with the theory of a dual origin of the APUD (amine content amine precursor uptake and decarboxylation) system (9 24 33)

Ellison and Neville (9) have reviewed the evidence concerning the relationship between the two proposed cell lines of the APUD system and their neoplastic counterparts According to this dual system the group of APUD cells of entodermal origin links with pancreatic islet cell tumours pituitary and parathyroid tumours carcinoid tumours of the gastrointestinal tract and the lung as well as oat cell carcinoma of the lung In contrast APUD cells of neuroectodermal origin correspond to phaeochromocytomas medullary carcinomas of the thyroid and multiple neurogenic neoplasms However Pearse (19 20) defining the APUD system on the basis of histochemical and cytochemical characteristics suggested a common progenitor cell in the neural crest Weichert (31) too suggested a common origin of the APUD system based on the functional capacity in the same histological type of tumour to produce a variety of peptide hormone substances He was of the opinion that the syndrome of MEA was simply a dysplasia of neural ectoderm

The existence of multiple endocrine tumours in individuals housing both groups of APUD cell lines seems to support the theories of Pearse and Weichert The present case report describes such a combination of mixed MEA In the literature moreover we have found seven cases representing both type 1 and type 2 in the same patient

CASE REPORT

A 65 year old woman was admitted to the hospital in Aug 1974 because of back pain weight loss and anorexia

Since the age of seven she had had generalized soft cutaneous tumours typical of Recklinghausen s disease In 1960 a fibrosarcoma was removed from the left breast and in 1968 the right thyroid lobe was ablated Microscopical examination revealed a typical medullary thyroid carcinoma Postoperatively the patient received radiotherapy (5 000 rads) to the neck region and substitution therapy was initiated with sodium L thyroxin

On the present admission the patient looked chronically ill Apart from the skin nodules hepatomegaly was the most notable finding on physical examination

Chest X ray showed enlargement of the right hilar region Alkaline phosphatase was 2 698 U/l (normal range 105–330) while serum lactic dehydrogenase glutamic oxaloacetic transaminase prothrombin bilirubin and electrolytes including calcium and phosphate were all normal The concentration of thyroid stimulating hormone in serum was moderately increased sub-
optimal substitution therapy with th
Hormonal findings also included a ex
cretion of 5 hydroxyindole acetic acid la



Fig 1 Medullary thyroid carc noma (×40)

m nes ketogen c sterods n the urine were also w th n the normal range Calc ton n in serum was moderately increased to ? ? ng/ml (normal 0 4–1 0) Liver scint gram (^{99m}Tc) demonstrated increased uptake in the r ght lobe Subsequent peritoneoscopy revealed hepatomegaly but no abnormal gross f nd ngs L ver biopsy fr m the right lobe performed during peritoneoscopy was normal

Bronchoscopy and med ast noscopy plus further en docr nolog c work up had been planned but the patient suddenly d ed ?1 days after adm ss on

At autopsy s ft grayi h wh te tumour t ssue was found to surround a small bronchus in the right upper lobe Med ast nal l mph nodes were enlarged f rm and wh t sh S m lar tumo r t ssue was found in the left dney as a nodule 3 cm in d ameter and n the upper troperitoneal pa e as a conglomerate of tumour tissue measuring 1?×1 11 cm surround ng the pancreat c head and part t h transverse colon

The t uc n he neck reg on showed scar format on and spar remnants of the thyro d gland were found One of the pa a hyro d glands was enlarged measuring 1×?×1 cm The r gh adrenal gland conta ned a yellow well def ned n dul mea uring 1×1 cm wh le the left one was normal Numerous small nodules in the sk n gave an appearan e f mul ple neurof bromatos s (von Reckl nghausen s d ea e)

On m croscopy the thyro d tumour removed in 1968 was found to cons st of irregular groups of tumour cells separated by a hyal ne amylo d pos t ve stroma The islands of tumour cell were of d fferent s ze cons st ng in some places merely of very small clusters and cords The cells had an eos noph l c granulated cytoplasm and were round or polyhedral The nuclei showed variat on in chromatin content in some places they were small and hyperchromat c in others b gger w th a coarse chromat n structure (F g 1)

The sk n and the t ssue of the neck sh wed changes l ke those of mult ple neurof bromatos s with sheets of fibroblasts and bundles of collagen The t ssue was loosely arranged and rather acellular The overly ng sk n con ta ned a normal number of melanocytes and p gmen laden macrophages

In the bronchus and surround ng lymph nodes the tumour tissue cons sted of rather small cells with round to oval nuclei and very scanty cytopla m The tumour cells invaded the bron hial mucosa and surrounded the cartilage (F g ?) The lymph nodes too were in aded by tumour cells S m lar tumour t ssue was observed n the small intest ne and in the kidney

The parathyro d glands were made up of small poly hedral cells closely packed with small un form nuclei Islands of oxyph l c cells were observed wh le no fat tissue was present The m croscop c appearan es were consistent w th parathyro d adenoma

Beneath its capsule the r ght adrenal gland showed large l p d laden cells of haphazard arrangement d f ferent from the normal gland There were no m toses and the nuclei were un form

DISCUSSION

The present patient had MEA involving f ve d f ferent neoplasms (Table I) Coexistence of neurofibromatosis and MEA has been documented several t mes in the literature (16 17 34) The occurrence of MEA_2 without phaeochromocytoma has been reported in some famil al cases (17) also in comb nation with neurogen c tumours (16) Thus our patient for instance definitely had a MEA syndrome related to the APUD system of neu roectodermal origin

Adenoma of the parathyro d has been related to both MEA_1 and MEA_2 (4 9 17 22 27 ?9 Supporters of the theory of a dual orig n of the

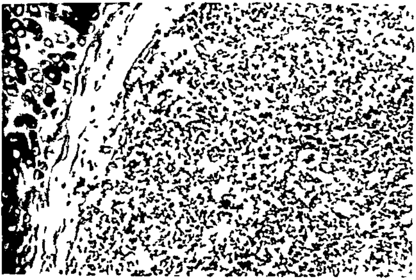


Fig 2 Small cell anaplastic carcinoma invading the bronchus (×100)

APUD system link the parathyroid adenoma with MEA_1 (9) It has been suggested that the parathyroid adenoma in MEA_2 may simply be reactive hyperplasia secondary to the secretion of thyrocalcitonin Reports of parathyroid adenoma in MEA_2 without evidence of medullary carcinoma of the thyroid seem to contradict this opinion (14) On the other hand the statements by Kaplan et al (13) and Voekel et al (30) that some phaeochromocytomas probably secrete thyrocalcitonin like substances support the opinion of reactive hyperplasia In our patient serum calcitonin was slightly increased so that the presence of parathyroid adenoma does not necessarily prove the existence of MEA_1 Similarly it has been claimed that catecholamine secretion from phaeochromocytomas may induce reactive hyperplasia of the parathyroid —at least in animals (11)

The capacity to secrete hormones belonging to the APUD system (3 9 18 31) as well as histo- and cytochemical similarities in the light and electron microscope relate the carcinoid of lung the carcinoid of gastrointestinal tract and small cell anaplastic carcinoma of the lung with MEA_1 to the APUD cell lines of entodermal origin (6 8 9 28) This correlation has also been established clinically in reports by several authors (10 12 21 25 26 32 35) The presence of small cell anaplastic carcinoma of the lung with widespread metastases in our patient is compatible with the existence of MEA_1

According to several authors (4 10 29) moreover non functioning adenomas of the adrenal cortex are a common finding in MEA_1 (38%) Therefore this finding in our patient presents another feature of MEA_1

Reviewing the literature we have been able to find seven other patients with tumour combinations belonging to both MEA_1 and MEA_2 (Table I) It is noteworthy that carcinoid of the gastrointestinal tract appears to be the predominant feature of MEA_1 and neurogenic tumours of MEA Neither carcinoids nor small cell anaplastic neoplasms of the lung have previously been reported as part of the mixed syndromes The finding of the latter tumour type however is not surprising since at least the lymphocytic subtype (oat cell) of small cell anaplastic carcinoma is most likely derived from the APUD cells as demonstrated by electron microscopy (6) This is furthermore the type of lung cancer which is most frequently found to be associated with the production of peptide hormone substances (23)

The incidence of this mixed syndrome is unknown It may have been overlooked since the carcinoids and the neurogenic tumours do not contribute to the usual features of MEA_1 and MEA

Whether or not the APUD system has a dual phylogenetic origin remains to be definitely established However the present case and the other seven cases of MEA of mix type from the

Table I *Cases of multiple endocrine adenomatosis (MEA) of mixed type described in the literature*

Reference no	Patients Age (y)	Sex	Tumour corresponding to APUD cell lines of entodermal origin (MEA_1)	Tumours corresponding to APUD cell lines of ectodermal origin (MEA_2)
5	13	♀	Duodenal carcinoid Adrenal cortical adenomata	Left adrenal phaeochromocytomas 2 paraaortic phaeochromocytoma
1	55	♂	Chromophobe adenoma of the pituitary Pancreatic islet cell tumours Adrenal cortical adenomas	Neurofibrosarcoma of the mediastinum
15	72	♂	Duodenal carcinoid	Multiple neurofibromatosis Left adrenal phaeochromocytoma
32	34	♀	Multiple duodenal carcinoids	Multiple neurofibromatosis
36	43	♀	Basophil adenoma of the pituitary Parathyroid adenoma	Medullary thyroid carcinoma Bilateral phaeochromocytomas
2	78	♀	Carcinoid of the intestine	Multiple neurofibromatosis
7	47	♀	Metastatic carcinoid of the intestine	Benign C cell adenoma of the thyroid
Present paper	65	♀	Small cell carcinoma of the lung Adrenal cortical adenomata Multiple adenomata of the parathyroids	Medullary thyroid carcinoma Multiple neurofibromatosis

literature support on clinical grounds the histochemical cytochemical and functional evidence of a common progenitor cell from the neural crests as the origin of the APUD cells

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Hemodynamic Influence of Multiple Congenital Arteriovenous Fistulas

Report of a Case

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ABSTRACT A 16 year old girl complained of sensations of weight, swelling warmth and frequent sweating from her right arm and hand Varicose veins were seen on the dorsal aspect of her right hand her right arm was longer than the left Since birth she had a capillary hemangioma involving her right shoulder Right arm and aortocervical arteriograms disclosed large arteriovenous fistulas Cardiac output was markedly increased No cardiac enlargement was seen at X ray examination The prognosis and future management of the patient are discussed

The association of hypertrophy subcutaneous varicose veins and naevus formations involving one extremity was described by Trelat and Monod in 1869 (7) In 1900 Klippel and Trenaunay (3) considered the simultaneous appearance of these changes important and described this clinical triad as "Naevus variqueux osteo-hypertrophique Unaware of the latter study Parkes Weber in 1907 (5) reported a few cases with the typical features described by Klippel and Trenaunay and others with clear indications of large arteriovenous communications The present paper introduces a patient with large arteriovenous communications in her right arm and shoulder The management of this young girl with but minor complaints has presented an interesting problem as the possibility of her developing cardiac failure must be considered

CASE REPORT

A 16-year-old girl 160 cm tall weighing 45 kg was referred to the Medical Out patient Department at Serafimerlasarettet in April 1975 with a chief complaint of swelling of the right hand Since birth she had had a non elevated birthmark on her right shoulder and the right lower lateral part of her neck Her mother s pregnancy and delivery had been without complications The family history was non contributory The patient had not suffered from any previous severe diseases There was no history of traumatism to arms or legs For as long as she could remember her right arm had been larger markedly stronger and warmer than her left She had also a tendency to increased sweating of the involved extremity and an eczematous reaction located in the palm and between the fingers She claimed that her right arm and the right upper parts of her chest were more sunburnt when exposed to the sun The symptoms from her right arm had increased during the last few years

Physical examination revealed an adolescent of healthy appearance A few varicose veins were seen on the dorsal aspect of her right hand which appeared grossly larger than her left Her right radial pulse was markedly stronger than the left The blood pressure in the right arm was 145/70 and 135/70 in the left Her right forearm and hand appeared to be more dusky and warmer than the left In the bend of the affected arm a thrill could be palpated and a loud bruit was heard on auscultation A weak systolic murmur of ejection type was heard with a maximum in the right second intercostal space No other bruits were heard over the chest The length of both arms was measured radiographically The right proximal part was 1 0 cm longer than the left while the distal part was 0 5 cm longer making a total difference in length of 1 5 cm

A venous plethysmography revealed a resting arterial flow of 58 5 ml/100 ml/min compared with 3 0 in the left arm Oscillometric indices were markedly increased in the right arm but normal in the left Digital BP was normal in the affected arm but showed a low amplitude in the left the difference in BP between the arm and the thumb being 10 and 30 mmHg in the right and left arms respectively

Aortocervical and arm arteriograms revealed a right subclavian artery with a diameter of 15 mm in the mid clavicular line This diameter was equal to the diameter of the midshaft of her humerus Her axillary artery was also enlarged The common carotid and vertebral arteries



Fig 1 Aortocervical arteriogram *LTA* = lateral thoracic artery *TAA* = thoracoacromial artery

were of ordinary size The superficial cervical the supreme thoracic the lateral thoracic and the thoracoacromial arteries were enlarged and tortuous Signs of arteriovenous communications to the axillary vein were noted from the latter two arteries (Fig 1) Shunting through networks of small arteries in the arm was seen from the dilated circumflex artery of the humerus the dorsal thoracic the recurrent ulnar and radial and the anterior interosseous arteries (Fig 2) Chest X ray was completely normal with a relative heart volume of 370 ml ECG and routine laboratory tests were normal

The total blood volume measured by [131] I albumin technique was within the normal range Cardiac output however was found to be 11 4 l/min as measured by the direct Fick method cardiac index (CI) 8 0 l/min When an arterial cuff applied proximally on the right arm was inflated to 300 mmHg her cardiac output decreased to 9 5 l/min (CI 6 6 l/min) indicating the presence of arteriovenous communications above the shoulder joint The difference in arteriovenous oxygen saturation was only 7% confirming the presence of large arteriovenous shunting Basal metabolic homeostasis was established and confirmed by oxygen consumption determinations

DISCUSSION

In a previous paper we have presented eight patients with one or more symptoms of the Klippel-Trénaunay triad (1) None of those patients had any angiographic signs of arteriovenous communications although arteriovenous shunts were found at the operation in subcutaneous and muscular tissues in two patients The present patient showed evident clinical signs of arteriovenous communications which were angiographically visualized She had all the features of the original Klippel-Trenaunay triad that is varicose veins osteohypertrophy and hemangioma However her main problem was of hemodynamic nature since she had gross arteriovenous shunting

Arteriovenous fistulas are known to increase the

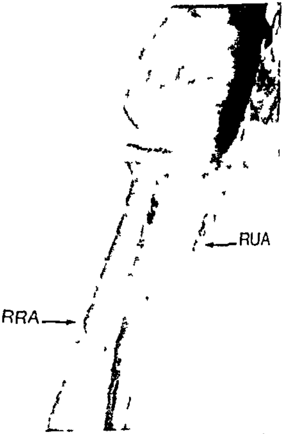


Fig 2 Arteriogram of the arm *RUA* = recurrent ulnar artery *RRA* = recurrent radial artery

heart rate stroke volume total cardiac output and systemic peripheral resistance leading to increased left ventricular stroke work (4) Depending on the size of the arteriovenous shunt or shunts and the passage of time left ventricular hypertrophy will appear and may eventually lead to congestive heart failure Besse et al (2) and Szilagyi et al (6) found that patients with congenital arteriovenous shunts were less liable to develop heart failure than those who have acquired shunts The latter authors point out that in the congenital shunts the communications are multiple tortuous and often traverse narrow branches This leads to an increased peripheral resistance while in acquired shunts the resistance is minimal

Our patient had multiple arteriovenous communications both distally and proximally to the shoulder joint She had no cardiac enlargement no symptoms from systemic circulation and only minor local complaints According to our experience the risk of developing venous incompetence with secondary cutaneous lesions is less pronounced when the upper rather than the lower extremity is involved The operative risk at this stage related to her complaints does not seem to warrant operative treatment If she develops cardiac enlargement and symptoms of left cardiac failure in the future however we feel that surgical intervention should be reconsidered

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ANNOUNCEMENTS

Second World Congress on Intensive Care will be held in Paris France Sept 19-23 1977 and will be devoted to the presentation of original works within the following fields

Acute respiratory failure cardiovascular failure acute renal failure neonatal and pediatric intensive care metabolic disorders gastrointestinal and nutritional disorders neurologic problems infectious problems traumatology (including burns dorwnings and thermic disorders) pharmacology toxicology experimental studies monitoring and computer applications problems involved in teaching organization and economy in intensive care Abstracts of non published original works should reach the Scientific Secretariat before Jan 31 1977

Information Scientific Secretariat Dr R Nedey Hôpital Foch 92151 Suresnes France

The International Prize for Modern Nutrition amounting to Sfr 15000 — will be awarded in Sept 1977 by the Central Union of Swiss Milk Producers Berne to a scientist from one of the following countries Argentina Australia Austria Belgium Brazil Bulgaria Canada Czechoslovakia Denmark Finland France India Ireland Israel Italy Japan Kenya Luxemburg the Netherlands New Zealand Norway, Poland South Africa Spain Sweden Switzerland United Kingdom USSR West Germany

The subject chosen is *Fermented milk products and nutrition* (biological clinical and epidemiological aspects) All scientists who have worked in this field are egligible

Applications Professor M Demole 4 chemin Castoldi CH 1208 Genève Switzerland until Feb 15 1977 with 3 copies of a) curriculum vitae b) list of works c) reprints of 2 or 3 papers on the theme of the prize published in the last 5 years (no typewritten papers) in English French or German

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Sarcoidosis of the Spleen

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ABSTRACT To determine the frequency of splenic involvement in sarcoidosis fine needle aspiration biopsies of the spleen have been performed on 77 patients with verified sarcoidosis Spleen size was normal in 71 patients and enlarged in 6 Splenic sarcoidosis was demonstrated in 53% of all patients and in 67% of those with and in 47% of those without known extrathoracic manifestations No complications occurred with this simple bedside procedure In sarcoidosis, fine-needle aspiration biopsy of the spleen is a valuable diagnostic tool especially when visible or palpable lesions are absent Its use is recommended before the use of mediastinoscopy bronchoscopy, lung biopsy or laparoscopy

Sarcoidosis is by definition a multisystem disease that most often affects the lymph nodes lungs eyes skin liver and spleen The diagnosis is acceptable for clinical purposes when a typical clinical picture is present in combination with a positive Kveim test and/or the presence of epithelioid cell granulomas in tissue specimens (Report of the medical group II International Conference on Sarcoidosis Washington D C 1960)

The reported frequency of splenomegaly in sarcoidosis has varied with the ethnic composition of the series In Finnish series consisting mostly of acute and subacute cases splenomegaly has been rare In one series of 140 patients only 2 (1 4%) had splenomegaly (12) In the USA where chronic sarcoidosis is more common the reported frequency of splenomegaly has ranged from 8% (8) to 40% (9) but has usually been about 20% (3 6 13) In English series splenomegaly has been noted in 11–31% of the patients (11 15)

Autopsy reports in cases of generalized sarcoidosis suggest that the spleen is the second most common site of involvement (2) and that a spleen of normal weight and size may be affected In their review of the autopsy findings in 138 cases of sarcoidosis Branson and Park (1) reported splenic involvement in 49 5%

Even though splenomegaly is considered rare in Finnish patients with sarcoidosis the fact that this is a multisystem disease invites investigation of the frequency with which the spleen is affected in different types of clinically proven sarcoidosis Furthermore if a simple and rapid bedside procedure could be used to demonstrate splenic involvement in a high proportion of patients with sarcoidosis this would be of diagnostic importance especially in patients without known extrathoracic manifestations

MATERIAL

The series consisted of 77 patients 53 women and 24 men with a mean age of 36 years Clinical data and diagnostic criteria are given in Table I All patients had intrathoracic lesions with radiographic findings compatible with sarcoidosis The diagnosis was confirmed in all cases by a Kveim test and/or the histological examination of tissue specimens (from peripheral or mediastinal lymph nodes bronchial or nasal mucosa liver skin salivary glands or lung parenchyma)

Extrathoracic sarcoidosis was found or excluded by careful inspection of the skin palpation of lymph node regions and salivary glands examination of the eyes and nose liver enzyme tests a percutaneous liver biopsy in patients with abnormal results in liver enzyme tests and the examination of any other suspected lesion or pathological finding that might have been caused by sarcoid involvement Extrathoracic manifestations were documented in 30 patients peripheral lymphadenopathy in 22 skin sarcoidosis in 6 a positive liver biopsy in 8 ocular lesions in 6 salivary gland involvement in 3 and bone cysts nephrocalcinosis and diabetes insipidus in 1 patient each

Table I *Clinical data of and diagnostic criteria used for 77 patients with sarcoidosis*

Chest X ray	No of pats	Women	Men	Diagnostic criteria: Kveim test[a]	Granulomas in tissue specimens[b]	Extra thoracic lesions	Splenomegaly
Stage I	39	26	13	24/28	28/33	3	1
Stage II	35	25	10	14/22	26/30	24	4
Stage III	3	2	1	0/1	3/3	3	1
Total	77	53	24	38/51	57/66	30	6

[a] No of positive tests/no of tests performed
[b] No of positive biopsies/no of biopsies performed

The size of the spleen was measured radiographically Only 6 patients had moderate splenomegaly the length of the organ exceeding 16 cm Of these patients one had a clearly palpable spleen and one had signs of hypersplenism

METHODS

The spleen was located by percussion The aspiration device consisted of a 0 7–0 8 mm needle on a disposable 10–20 ml syringe usually with a Luer adaptor The needle was inserted into the tenth intercostal space 3–5 cm dorsally of the mid axillary line During aspiration patients were asked to catch their breath in mid-expiration Although not necessary local anaesthesia was used in a few instances The only contraindications for fine needle aspiration were an overt bleeding diathesis or a platelet count below 100 000/mm³

The aspirated material was spread on glass slides air dried and stained with May-Grünwald-Giemsa The aspirated material was usually sufficient for cytological examination if not the aspiration was repeated The aspiration was not repeated if sufficient material was obtained but was negative with respect to granulomatous changes

RESULTS

The cytological finding in a splenic fine needle aspirate was interpreted as consistent with sarcoidosis if groups of epithelioid cells were detected (Fig 1) The presence of giant cells of Langhans type was not considered mandatory for the diagnosis Such cells were however found in 9 of the 41 aspirates classified as positive If an aspirate contained only a few scattered epithelioid cells it was classified as negative

The results of all splenic fine needle aspirations are shown in Table II A cytological finding consistent with sarcoidosis was found in 53% of the patients studied In patients with known extrathoracic manifestations of sarcoidosis the frequency of positive aspirates was 67% and in those without signs of extrathoracic lesions 47%

Of the 36 patients in whom bilateral hilar lymphadenopathy was the only sign of sarcoidosis splenic involvement was detected in 17 Of the six patients who had radiographically demonstrable splenomegaly five also had splenic sarcoidosis

All splenic fine needle aspirations were performed without complications

DISCUSSION

Patients with chronic sarcoidosis frequently have splenomegaly In most cases the splenomegaly is asymptomatic but it can sometimes cause abdominal discomfort Rupture of the spleen (10) or hypersplenism (5) may complicate the course and splenectomy has been required (4) In Finland most cases of sarcoidosis have a favourable prognosis

Table II *Results of fine needle aspiration biopsies of the spleen in 77 patients with sarcoidosis*

Chest X ray	Extra thoracic manifestations	No of pats	Splenic sarcoidosis detected: No of pats	%
Stage I	Not noted	36	17	47
Stage I	Present	3	2	67
Stage II	Not noted	11	5	45
Stage II	Present	24	16	67
Stage III	Present	3	1	33
Total		77	41	53

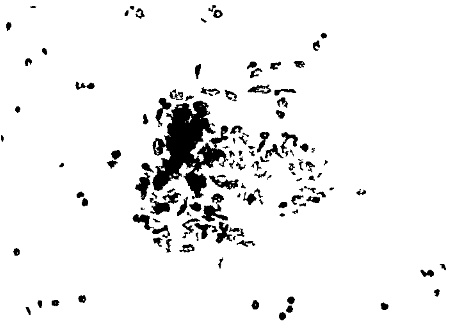


Fig 1 Epithelioid cell granuloma in a splenic fine needle aspirate from a patient with sarcoidosis and with a spleen of normal size

and the signs of the disease usually disappear with or without treatment within 12–24 months (12) In these patients splenomegaly is rare It was radiographically demonstrated in 8% of the patients in this series but only one had a clearly palpable spleen

The results of this investigation suggest however that in Finnish patients with sarcoidosis involvement of the spleen is fairly common an observation that conforms with the known multisystem nature of the disease Of the patients with bilateral hilar lymphadenopathy as the only sign of sarcoidosis splenic involvement was demonstrated in almost 50% This also seems to show that grouping patients with sarcoidosis into those with and those without extrathoracic lesions is meaningless Extrathoracic manifestations can only be ruled out by laparotomy and splenectomy procedures that are not necessary in sarcoidosis

The fine needle aspiration biopsy is a simple procedure shown to be useful in the diagnosis of various parenchymal and tumorous disorders (16) The absence of complications in this series conforms with previous reports on the safety of splenic fine needle aspiration biopsies when performed on patients with normal platelet counts Soderstrom recently reported on the performance of more than 1000 splenic fine needle aspirations during a 10-year period without a single complication (17)

The demonstration of epithelioid cell granulomas in biopsy specimens is almost always desirable to confirm the clinical diagnosis of sarcoidosis (7) The histological finding is not however pathognomic for sarcoidosis other granulomatous disorders must be excluded In patients without visible or palpable lesions biopsies of the bronchial mucosa mediastinal lymph nodes lung parenchyma or liver are those most commonly performed Mediastinoscopy and thoracotomy give positive results in almost 100% of the cases but require general anaesthesia Percutaneous needle biopsy of the lung parenchyma also gives positive results in more than 90% but is not a bedside procedure and complications have been reported (18) The reliability of bronchial biopsies in patients with pulmonary infiltrations is about 70% (14) The reliability of percutaneous liver biopsies is about 60% and the frequency of positive findings may rise to 80% if the specimen is taken at laparoscopy (19)

The splenic fine needle aspiration biopsy is a simple safe bedside procedure that seems to be a reliable alternative to all the above mentioned procedures as the initial method for demonstrating granulomatous changes in sarcoidosis

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Observations on the Different Calcium Metabolic Patterns in Sarcoidosis

A Metabolic and Kinetic Study

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ABSTRACT Combined calcium balance and 47Ca turnover studies in sarcoidosis (4 patients) and vitamin D intoxication (1 patient) disclosed three different patterns of calcium metabolism One patient with sarcoidosis had a normal metabolism of calcium, and two patients presented the usual pattern of intestinal hyperabsorption, hypercalcemia, and hypercalciuria The fourth patient with sarcoidosis and the patient with vitamin D intoxication, both studied during spontaneous remissions, presented the third pattern The main features here were hypercalcemia *despite* normal intestinal absorption of calcium, enlarged exchangeable calcium pool, accelerated accretion and resorption rates, hypercalciuria, and a distinctly negative calcium balance This pattern of remission seems to represent a mobilization of extraosseous or metastatic calcifications, rather than a resorption of bone calcium

Hypercalcemia (18) and hypercalciuria are well known complications of sarcoidosis although their frequency varies considerably averaging some 10% (14) and 50% (30) respectively A few investigators have attributed the hypercalciuria to either a primary decrease in the tubular reabsorption of calcium (TRCa) (28) or to excessive bone resorption (21) while the majority ascribe it to an increased intestinal absorption of calcium (1 4 23) According to this hypothesis (44) hyperabsorption raises plasma ionized calcium (15) leading to suppression of parathyroid hormone (PTH) secretion (9 47) and consequently a decrease in the TRCa (30 45) This sequence of events appears to result from an increased sensitivity to vitamin D (1 4 10 31) This hypersensitivity may possibly involve the skeleton as well as the intestinal tract (3)

Along with our studies of the renal handling of calcium in hypercalcemic disorders reported previously (45 48) we performed traditional calcium and phosphorus balance and 47Ca kinetic studies in four different types of patients 1) normocalcemic active sarcoidosis 2) hypercalcemic active sarcoidosis 3) hypercalcemic sarcoidosis in spontaneous clinical remission and 4) vitamin D intoxication in remission

Our observation of two different calcium metabolic patterns in hypercalcemic sarcoidosis apparently related to the stage of disease forms the basis of a hypothesis unifying the observations reported hitherto

MATERIAL

Five patients were studied A summary of their clinical data is given in Table I

Case reports

Patient 1 A 54 year-old female with arthralgias myalgias and intermittent fever for about two years Erythema nodosum was found on admission Chest X ray was normal but hepatic musculo-cutaneous and tonsillar biopsies showed epithelioid granulomatous lesions No abnormalities of the calcium metabolism could be demonstrated

Patient 2 A 58 year-old female with conjunctivitis parotid gland enlargement fatigue and weight loss for two years Uveitis peripheral lymphadenopathy disseminated pulmonary infiltration hypercalcemia and

Preliminary data presented in part at the 4th International Sarcoidosis Conference in Paris Sept 1966

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Table I *Patient material*

Pat no	Sex	Age (y)	CCr (ml/min)	S-Ca (mg/100 ml)	Diagnosis and duration	Medication
1	♀	54	72	9 4	Sarcoidosis 2 y	None
2	♀	58	33	11 2	Sarcoidosis 2 y	None
3	♂	25	90	11 7	Sarcoidosis 5 y	None
4	♂	26	77	10 9	Sarcoidosis horseshoe kidney 6 months	None
5	♂	44	25	11 5	Postsurgical hypoparathyroidism vitamin D intoxication months	AT 10 discontinued 4 weeks before study thyroidin II×2 daily

moderate renal insufficiency were found on admission Bone marrow and lymph node biopsies confirmed the diagnosis of sarcoidosis Neither band keratopathy nor X ray-demonstrable renal calcifications were found A renal biopsy was unsuccessful Treatment with prednisone was still necessary 3 years after the balance study

Patient 3 A 25 year-old male with a history of sarcoidosis of 5 years duration Uveitis and sialoadenitis had been present and on admission he revealed marked generalized peripheral lymphadenopathy enlarged hilar lymph nodes disseminated pulmonary infiltrations and hypercalcemia X ray-demonstrable renal calcifications and band keratopathy were not present The diagnosis of sarcoidosis was confirmed by hepatic and lymph node biopsies while a renal biopsy was unsuccessful After the balance study treatment with prednisone was initiated It was still necessary nearly 3 years later

Patient 4 A 26-year-old male with sarcoidosis presenting by acute renal insufficiency 6 months prior to the balance study Spontaneous improvement in renal function ensued gradually and during the metabolic study slight uveitis hilar lymphadenopathy and a modest hypercalcemia formed the symptomatology Hepatic musculo-cutaneous and renal biopsies revealed epithelioid

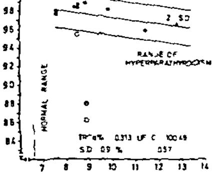


Fig 1 Tubular reabsorption of calcium (*TRCa %*) related to serum ultrafiltrable calcium in hyperparathyroidism (●) and in patients with sarcoidosis (O) or vitamin D intoxication (◯)

granulomas *Even though renal calcifications could not be demonstrated by X-ray the kidney biopsy specimen contained heavy calcium deposits* After the balance study (in Dec 1965) the activity of the disease as evaluated clinically and biochemically gradually abated and since March 1966 all laboratory tokens of sarcoidosis have vanished except for a minimal hypergammaglobulinemia (1 4 g/100 ml)

Patient 5 A 44 year-old male who in 1959 underwent thyroidectomy because of thyrotoxicosis One month later tetany occurred (serum calcium 5 9 mg/100 ml) and treatment with AT 10 and thyroidin was initiated During 1965 symptoms of hypercalcemia and renal insufficiency developed Renal calcification could not be demonstrated by X ray and unfortunately renal biopsy was unsuccessful AT 10 was discontinued 4 weeks before initiation of the balance study Hypercalcemia gradually abated together with a spontaneous improvement in renal function during the following 4 months Later on hypocalcemia and tetany redeveloped confirming the original diagnosis of postsurgical hypoparathyroidism

Control subjects

The data on tubular reabsorption of phosphorus (TRP%) and TRCa% in chronic renal failure and in primary hyperparathyroidism derive from a previous publication (48) Eleven female and 15 male healthy volunteers served as control subjects in the estimation of the normal TRP% level on the given diet (approximately 1100 mg phosphorus/day)

METHODS

The patients were subjected to a metabolic balance regime according to the principles advocated by Reifenstein et al (39) After 5–11 days on a constant diet collections of feces and urine were started

Diet was given in a repetitive 3-menu system On consecutive days during each 6-9-day period a pl bucket was fed the same amount of food and fluids a the patient The contents hereof were analyzed calcium and phosphorus the measured levels of which are given in Table III The diet of patient 1 was analyzed for phosphorus but the calculated was similar to that of the other patients In all patier

Table II *Biochemical characteristics*

Pat no	Serum: Calcium (mg/ 100 ml)	Serum: Phosphorus (mg/ 100 ml)	Serum: Alkaline phosphatases (U/100 ml)	Serum: Standard bicarbonate (mEq/l)	Serum: UFCa (mg/ 100 ml)	Urine: CCr (ml/ min)	Urine: TRCa (%)	Urine: TmG/ GFR	Urine: TRP%
1	9 4	3 9	60	24 3		72			
2	11 2	3 5	26	24 2	8 90	33	85 8	1 19[b]	43 5
3	11 7	3 1	41	24 0	9 40	84	95 3	1 59[b]	76 8
4	10 9	4 5	47	25 0	8 34	72	95 9	1 32[b]	80 6
5	11 5	3 9	24	28 8	8 85	23	87 9	1 49[c]	40 4
Reference range (mean±2 S D)	8 7–10 6[a]	2 5–4 6[a]	13–38	21 3–25 8[a]	6 55–7 65			1 68–2 44	

[a] Data from Hornum (24) [b] Transbol and Halver (47) [c] Halver (16)

the dietary intakes of protein and sodium were kept constant at levels of about 1 g/kg b wt and 70–90 mEq/day respectively

Urine was collected in 24 hour portions and *feces* in periods of 5–9 days duration usually 6 days The number and lengths of individual periods for each patient are given in Table III

During the study all patients were kept ambulatory at a constant degree of indoor mobilization with total exclusion of direct sunlight Further details of the balance regime are given in a previous paper (24)

Chemical methods

Analysis for calcium and phosphorus in serum urine diets and feces was carried out according to methods published earlier (24) For technical reasons phosphorus was not analyzed in feces urine and diet in patient 1 and in feces in patient 3 Creatinine in serum and urine alkaline phosphatases and standard bicarbonate were analyzed according to the methods of Hawk et al (19) Bessey et al (5) and Siggaard Andersen et al (41)

Tracer technique and treatment of isotopic data

During the balance regime 47Ca chloride was given in an i v dose of approximately 20 μCi specific activity about 100 mCi/g Ca Samples of serum urine and feces were counted on the following 6–8 days determining the decay curve for 47 Ca in serum and urine and the cumulative loss of activity in urine and feces Details of preparation and kinetic analysis have been published earlier (24 The 2 compartment model of Wendeberg (49) was used in the calculation

Renal handling of calcium phosphorus and glucose

The 24 hour TRCa% and TRP% were calculated according to the formulas TRCa%=100×(1–(urinary calcium mg/24 h) × (serum creatinine mg/100 ml) (UFCa mg/100 ml)×(urinary creatinine mg/24 h)) and TRP%=100×(1–(urinary phosphorus mg/24 h)×(serum creatinine mg/100 ml)/(serum phosphorus mg/100 ml) × (urinary creatinine mg/24 h)) Serum ultrafiltrable calcium (UFCa) was analyzed as reported earlier (46)

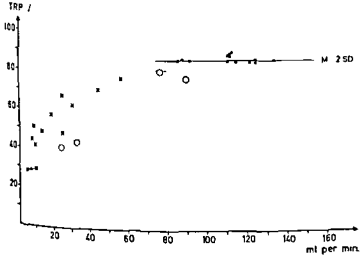


Fig 2 Tubular reabsorption of phosphate (*TRP* %) with varying clearance of creatinine in controls (●) patients with renal disease (■) hypercalcemic sarcoidosis (O) and vitamin D intoxication (O)

Table III *Balance data*

Pat no	Period*	Calcium (mg/24 h) Diet	Feces	Urine	Balance	Phosphorus (mg/24 h) Diet	Feces	Urine	Balance
1	I (8)	926	686	231	+9				
	47Ca II (5)	902	683	230	−11				
2	I (6)	839	301	560	−22	1 098	252	914	−68
	II (9)	839	315	611	−87	1 098	199	1 011	−112
	47Ca III (6)	839	372	550	−83	1 098	220	892	−14
3	I (6)	1 024	585	478	−39	1 179		955	
	47Ca II (6)	1 121	638	535	−52	1 179		971	
	III (6)	1 136	667	534	−65	1 179		868	
4	I (6)	765	579	422	−236	1 172	301	1 017	−146
	II (6)	780	631	392	−243	1 172	357	945	−130
	47Ca III (6)	826	595	393	−162	1 172	303	917	−48
	IV (6)	790	578	296	−84	1 172	271	902	−1
5	I (6)	784	622	395	−233	1 094	440	839	−185
	47Ca II (6)	784	679	352	−247	1 094	487	808	−201
	III (6)	784	814	345	−375	1 094	515	863	−284

* Length (days) within parentheses

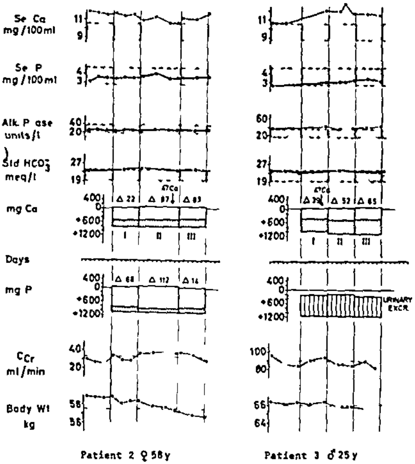


Fig. 3. Results of balance studies in active hypercalcemic sarcoidosis. Beside the serum levels of calcium, phosphorus, alkaline phosphatase and standard bicarbonate the figure indicates the levels of creatinine clearance (C_{Cr}), body weight and balances of calcium and phosphorus. The fecal (hatched) and urinary (blank) excretions per day of the two elements are charted according to Reifenstein et al (39).

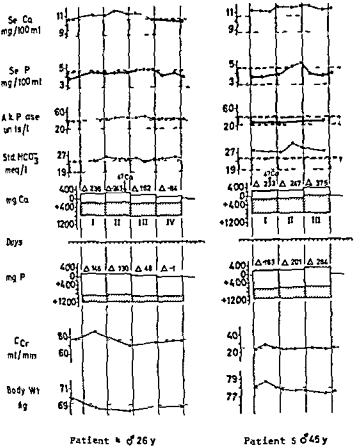


Fig 4 Results of balance studies in hypercalcemic sarcoidosis and vitamin D intoxication in spontaneous remission Beside the serum levels of calcium phosphorus alkaline phosphatase and standard bicarbonate the figure indicates the levels of creatinine clearance (C_C) body weight and balances of calcium and phosphorus The fecal (hatched) and urinary (blank) excretions per day of the two elements are charted according to Reifenstein et al (39)

The determinations of the maximal tubular reabsorptive capacity of glucose (TmG/GFR) were carried out by the method of Halver (16)

RESULTS

Blood chemistry

The calcium levels were normal in patient 1 and moderately increased in the others Serum phosphorus was normal in all (Table II Figs 3 and 4)

Renal handling of calcium glucose and phosphorus (Table II)

The glomerular filtration rate as measured by the clearance of creatinine (CCr) was reduced in two patients (nos 1 and 5) and had been so prior to the study in patient 4 The values of *TRCa%* which have been published earlier (48) are depressed compared with those of patients with primary hyperparathyroidism having a similar degree of hypercalcemia (Fig 1) Also with respect to *TmG/GFR* all values of the hypercalcemic patients are subnormal indicating a state of non parathyroid hypercalcemia (16 17 47) Despite this all TRP% values in the hypercalcemic patients are low and even below the average TRP% in ordinary renal insufficiency without hypercalcemia (Fig 2)

Cortisone tests

Administration of cortisone acetate 50 mg 3 times daily for 10–14 days to patients 2 and 3 led to a complete and rapid normalization of serum calcium (Fig 7 in Transbøl et al (48)) This evidence also points towards a true non parathyroid origin of the hypercalcemia (12)

Balance data (Table III)

While patient 1 was in balance for calcium patients 2 and 3 were both in a slightly negative balance for calcium and (for pat 2) for phosphorus too (Fig 3) Patients 4 and 5 were in a markedly negative balance for calcium and a negative balance for phosphorus (Fig 4)

Table IV *Kinetic data*

Pat no	Exchangeable pool (g)		Accretion (g/24 h)	Resorption (g/24 h)	Intestinal absorption ("true")		Endogenous fecal calcium (g/24 h)
	Rapid	Slow			g/24 h	%	
1	2 122	0 978	0 325	0 336	0 304	33 7	0 089
2	2 500	2 270	0 462	0 545	0 576	68 7	0 109
3	2 871	3 436	0 506	0 558	0 612	54 6	0 130
4	2 940	4 770	1 187	1 349	0 390	47 2	0 159
5	4 000	4 178	1 070	1 316	0 247	31 5	0 142
Reference range	2 20±0 45[b]	2 38±0 79[b]	0 449±0 101[b]		8 mg±2 mg/kg[c]	20-60[d]	0 167±0 032[b]

[a] Data from Hornum (24) [b] Mean±1 S D [c] Mean±2 S D [d] Range

Kinetic data (Table IV)

The exchangeable pool is considered to consist of one rapidly and one slowly exchangeable pool (E_I and E_{II}) Patient 1 with *normocalcemic sarcoidosis* had a low E_{II} while patients 2 and 3 had normal to slightly increased values and patients 4 and 5 very high values *Accretion and resorption* rates were normal in patients 1 2 and 3 but definitely increased in patients 4 and 5 *The intestinal adsorption* normal in patient 1 was high in patients 2 and 3 (68 7 and 54 6% respectively) and normal in patients 4 and 5 *Endogenous fecal calcium* was normal in all patients ranging from 0 089 to 0 159 g/24 h

DISCUSSION

With depressed levels of TRCa% (30 45 48) and TmG/GFR (16 17 47) sensitivity to cortisone (7 48) and unmeasurable concentrations of serum PTH (9) the hypercalcemia of sarcoidosis is characterized almost invariably as being of non parathyroid origin Nevertheless the occasional

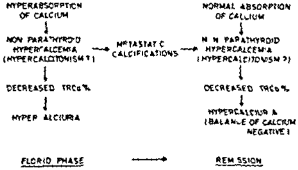


Fig 5 Hypothesis on pathophysiological patterns in hypercalcemic sarcoidosis

coexistence of hyperparathyroidism and sarcoidosis (11 25 32 47) necessitates a decision as to whether a non parathyroid or parathyroid hypercalcemia or possibly both is present In the present cases of hypercalcemic sarcoidosis and of vitamin D intoxication low levels of TRCa% and TmG/GFR and normalization of serum calcium either spontaneously (pats 4 and 5) or during cortisone administration (pats 2 and 3) provide the evidence for a true non parathyroid hypercalcemia In abundance patient 5 had postsurgical hypoparathyroidism Therefore if the TRP% was a valid measure of parathyroid function high values might be expected in these conditions Yet this was not the case Instead of being elevated TRP% values were all *below* the normal average for the present levels of CCr (Fig 2) This implies that other factors must be more important than PTH in determining the renal handling of phosphorus One such factor might be calcitonin which is released during hypercalcemia and possesses a phosphaturic action (42)

Several balance studies have characterized the metabolic disturbance as one of intestinal hyperabsorption of calcium (1, 4 23) Further it is recognized that the urinary excretion of calcium is often larger than one would expect from the intestinal absorption alone resulting in a negative calcium balance and thus implying other abnormalities of calcium metabolism than hyperabsorption (3 21 23) Some investigators interprete this pattern as an example of vitamin D intoxication in sarcoidosis attributing the negative calcium balance to teolysis (3 21) Admittedly administration of small doses of vitamin D (3 4 21) and exposure of sarcoid patients to ultraviolet light (10 31 43 exaggerate as the depletion of vitamin D amelio-

rates the disturbance of calcium metabolism (21) but the relevance of this for the pathogenesis of sarcoid hypercalcemia remains questionable Thus it has been shown that vitamin D depletion and administration of small doses of vitamin D also affect calcium metabolism in primary hyper parathyroidism (33) So far observations on the metabolism of vitamin D_3 in sarcoidosis have re mained contradictory (2 36)

Our studies were designed to examine whether a difference in the calcium metabolic pattern can be found in patients with normocalcemic sarcoidosis florid hypercalcemic sarcoidosis and hypercalcemic sarcoidosis in spontaneous remission This seems to be the case Patient 1 with normocalcemia demonstrates a normal pattern of calcium metabo lism Patients 2 and 3 with a florid hypercal cemic sarcoidosis which after the end of study required steroid treatment for years are both hyperabsorbers of calcium with a slightly negative balance of calcium Patient 4 in spontaneous re mission has a normal absorption of calcium but a distinctly negative balance because of a hyper calciuria in excess of intestinal absorption Patient 5 with vitamin D intoxication showed essentially the same pattern of calcium metabolism as patient 4 the sarcoid patient in remission As treatment with AT 10 was discontinued 4 weeks before study he naturally presented a state of remission like patient 4 (Fig 4)

In view of this pattern we do not find it reason able to regard the excess hypercalciuria as an expression of bone resorption or osteolysis due to vitamin D If a vitamin D action were responsible for the high urinary excretion of calcium a paral lelism should be expected between the degree of hypercalciuria and the intestinal absorption This was not the case Patient 4 with a normal in testinal absorption of calcium had the most pro nouncedly negative balance

As an alternative hypothesis we suggest the pos sibility that the excess hypercalciuria is due to mo bilization of metastatic calcifications for the following reasons 1) In hypercalcemic sarcoidosis as in vitamin D intoxication metastatic calcifica tions are frequently found (7 8 27 40) They are often invisible on X rays but recognizable by renal biopsy—as in patient 4 (6 34) and by the renal insufficiency which is present almost inevitably (26 34 48) These calcifications may be reversible during dietetic manipulation (13) like those of tumoral calcinosis (37) 2) As stated by Heaney (20) the accretion and resorption rates are not identical with osseous turnover alone but do in fact also include the rate of exchange in non osseous calcified tissues This is demonstrated by the findings of increased pool size and accretion/ resorption rates in conditions with recognizable metastatic calcifications such as the milk alkali syndrome (22 38) vitamin D intoxication (22) and most evidently tumoral calcinosis (29) In the latter condition an exchange of calcium between tumoral masses and serum was found which greatly exceeded the exchange between bones and serum 3) In hypercalcemic sarcoidosis the few reported tracer studies have shown increased pool size and accelerated accretion/resorption rates (3) Our patients had large pools and raised accretion/ resorption rates patient 4 having a distinct in crease 4) There is no evidence suggesting a rela tion between X ray demonstrable bone affection and hypercalcemia in sarcoidosis (35) 5) A mo bilization of metastatic calcifications is recognized as a potentially hypercalcemizing condition (24)

On the basis of this evidence we find it reason able to conclude that during the remission of hyper calcemic sarcoidosis and vitamin D intoxication resorption of metastatic calcifications may be responsible for the high urinary excretion of cal cium in excess of the intestinal absorption

In summary the results of our studies and those reported in the literature state that two different disturbances of calcium metabolism exist in hyper calcemic sarcoidosis Our studies suggest that the disturbance is dependent on whether the disease is in a state of climax or remission In *periods of activity* the pattern of calcium metabolism is that of hyperabsorption of calcium hypercalcemia and hypercalciuria In *periods of remission* hyperab sorption ceases but the hypercalcemia and/or the hypercalciuria is maintained possibly for months by mobilization of reversible metastatic calcifica tions thus leading to a distinctly negative calcium balance (Fig 5)

ACKNOWLEDGEMENTS

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1-Alpha-hydroxycholecalciferol-induced Changes in the Renal Handling of Phosphate and the Serum Parathyroid Hormone Level

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ABSTRACT The effect of 1 alpha hydroxycholecalciferol (1α OH D_3) on the renal handling of phosphate and the immunoreactive parathyroid hormone in serum (i PTH) has been studied in 10 patients with a wide range of glomerular filtration rate (GFR), maximal tubular reabsorption of phosphate (TmP) and i PTH The patients were treated with 2 µg 1α OH D_3 per day for approximately 80 days Before and after this period of treatment the TmP i PTH ^{51}Cr EDTA clearance, extracellular volume standard bicarbonate, and serum calcium were measured in each patient The TmP/GFR ratio was used as an index of the renal handling of phosphate The index increased significantly (mean 76 5%, $p<0\ 01$) during the treatment, while i PTH decreased significantly (mean 37 0%, $p<0\ 01$) An inverse significant correlation was demonstrated between TmP/GFR index and i PTH both before ($r=-0\ 87$ $p<0\ 001$) and after ($r=-0\ 79$, $p<0\ 01$) the administration of 1α OH D_3 while none of the other factors investigated were correlated to the index It is concluded that 1α OH D_3 increases the TmP/GFR index and reduces i PTH in a parallel manner and it is therefore suggested that the 1α OH D_3-induced changes in the renal handling of phosphate may be explained as being mediated solely via the suppression of i PTH

Although the parathyroid function has a key role in the regulation of the renal handling of phosphate (1 8 24) it is evident that other factors are or may be involved as well in this important homeostatic mechanism These factors are primarily the acid-base balance (21) the extracellular volume (15) the circadian rhythm (19) steroids (16 23) diuretics (7) ionized calcium (3) and possibly vitamin D (22) The importance of the parathyroid hormone was stressed in our previous investigation (14) where an inverse significant correlation was demonstrated between the serum concentration of immunoreactive parathyroid hormone (i PTH) and the renal handling of phosphate while the latter did not correlate with either the extracellular volume standard bicarbonate or serum calcium The renal handling of phosphate was expressed as the ratio between the maximal tubular reabsorption of phosphate (TmP) and the glomerular filtration rate (GFR) since this is the most consistent index (4 25)

The role of vitamin D in the tubular reabsorption of phosphate which has been a matter of controversy (17 22) is further examined in the present report 1 Alpha hydroxycholecalciferol (1α OH D_3) a crystalline vitamin D analogue which is converted in the liver (27) to the genuine active vitamin D (10 20 26) has recently become available (11) The present study was undertaken to elucidate the effect of 1α OH D_3 treatment on the renal handling of phosphate and on the parathyroid function

MATERIAL

The material was fundamentally the same as in our previous report (14) except that 5 patients had to be omitted (2 had initiated hemodialysis 1 received intensive steroid treatment 1 was hypercalcemic and 1 died after a cerebral hemorrhage) So the material consisted of 10 patients (5 females 5 males) aged 22–58 years (mean 38) with creatinine clearances of 4–65 ml/min (mean 39 6) Six patients (3 females 3 males) aged 22–58 years (mean 36) had well functioning kidney allografts with creatinine clearances of 43–65 ml/min (mean 59 1) The average time of the present investigation after the kidney transplantation

was 18 months (range 6–60) The transplanted patients received a daily prednisone dosage of 7 5–25 mg (mean 15 0) which was unaltered throughout the study None of these patients showed clinical or biochemical signs of rejection during the study Four patients (2 females 2 males) aged 23–50 years (mean 40) had varying degrees of chronic renal insufficiency with creatinine clearances of 4–20 ml/min (mean 10 4) The nephrological disease in 2 patients was chronic interstitial nephropathy in 1 patient chronic glomerulonephritis and in 1 polycystic kidney dis ease No patient received dialysis treatment 5 patients received diuretic treatment which was withdrawn 24 hours prior to the study

METHODS

The patients received 1α-OH D_3 dissolved in propylene glycol orally for approximately 3 months (mean 80 days) The dose of 1α OH D_3 was 2 μg/day but was reduced if hypercalcemia (serum calcium exceeding 2 7 mmol/l) de veloped The 10 patients served as their own controls All investigations were carried out before and after treatment with 1α OH D_3 (Leo Pharmaceuticals Copenhagen)

The methods detailed below were as previously de scribed (14) The investigations were carried out at 9 00 a m The 6 kidney transplanted patients who were normo- or slightly hypophosphatemic (serum phosphate 0 72–1 04 mmol/l) at the start of the study received an i v phosphate infusion in order to obtain maximal tubular reabsorption of phosphate The infusion consisted of a 0 1 M solution of phosphate buffered at pH 7 4 and was deliv ered at a rate of 100 ml/h with an infusion pump (Braun Infusomat®) After 60 min the 6 patients had serum phos phate values (mean 1 80 mmol/l) close to the 4 uremic patients (mean 2 06 mmol/l) At that time the tubular reabsorption of phosphate could be considered as maxi mal in all 10 patients During continuous phosphate infu sion in the transplanted patients and without phosphate administration in the uremic patients a urine sample was collected in the following 120-min period (All patients could void on request) This 120-min period was repeated as a control in 8 of the 10 patients

Blood samples were collected every 30 min during the urine sampling period and analyzed for phosphate The average serum phosphate was used in the calculations At the beginning of the period of urine sampling a blood sample was collected and analyzed for i PTH serum calcium and standard bicarbonate GFR was estimated during the study using the single injection technique of ^{51}Cr EDTA (18) and similarly the extracellular volume was calculated as the ^{51}Cr EDTA distribution space (12) The analysis of i PTH in serum was performed by the Stock holm Immunlaboratory AB (2) Normal range 1 1–2 5 ng bovine PTH equivalents/ml Phosphate in plasma and urine was measured as described by Dryer et al (6) All phosphate values were expressed as mmol/l The TmP (μmol/min) was calculated as the difference between filtered (GFR×mean serum phosphate) and excreted phosphate (urine phosphate×urine volume/min) Finally the TmP/GFR index (μmol/ml) was calculated

RESULTS

The reproducibility of the estimation of TmP in a 120-min period was tested by duplicate measure ments in 8 of the 10 patients The technique wa found to be reproducible with a coefficient of varia tion of 12%

The values for TmP/GFR index i PTH an serum calcium as determined in the 10 patients be fore and after 80 days of treatment with 1α OH D are given in Table 1 The TmP/GFR values varie over a wide range (0 03–0 85 μmol/ml) prior to th treatment and a significant (p<0 01) increase (mea 26 5%) was seen after the treatment The i PTH values varied similary over a wide range (1 9–13 ng/ml) and decreased (mean 37 0%) significantl (p<0 01) after the treatment Serum calcium in creased by 4 5% (p<0 05) while the extracellula volume and standard bicarbonate remained un changed

Before the administration of 1α OH D_3 an in verse significant correlation (y=−11 60x+10 21 r=−0 87, p<0 001) was found between th TmP/GFR index and i PTH while the extracellula volume and the serum concentrations of standard bicarbonate and calcium did not correlate to the index After 80 days of treatment with 1α OH D_3 an inverse significant correlation (y=−5 00x+5 97 r=−0 79 p<0 01) persisted between the same two parameters The slopes and intercepts of these two

Table 1 *TmP/GFR i PTH and serum calcium be fore (B) and after (A) 80 days of treatment with 1α OH D_3*

Pat no	TmP/GFR (μmol/ml) B	TmP/GFR (μmol/ml) A	i PTH (ng/ml) B	i PTH (ng/ml) A	Serum calcium (mmol/l) B	Serum calcium (mmol/l) A
1	0 85	0 92	2 1	2 0	2 60	2 67
2	0 77	0 84	1 9	2 2	2 39	2 44
3	0 72	0 80	2 4	2 3	2 68	2 54
4	0 69	0 77	2 8	1 2	2 41	2 64
5	0 58	0 65	3 3	2 3	2 48	2 72
6	0 42	0 71	3 0	1 9	2 49	2 51
7	0 38	0 58	4 3	3 2	2 67	2 65
8	0 35	0 39	3 5	3 0	2 08	2 47
9	0 11	0 32	9 5	6 3	2 38	2 54
10	0 03	0 26	13 0	4 1	2 27	2 41
Mean change (%)	26 5 ↑		37 0 ↓		4 5 ↑	
p value	<0 01		<0 01		<0 05	

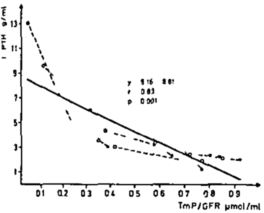


Fig. 1. The correlation between TmP/GFR and i PTH in 10 patients before (O) and after (●) 80 days of treatment with 1α OH D_3. --- = individual course of each patient.

...ines did not differ significantly (5). Con... an inverse significant correlation ($y = -9.16x+8.81$, $r=-0.83$, $p<0.001$) could be demonstrated between the TmP/GFR index and i PTH in the 10 patients whether or not they received 1α OH D_3. This regression line is reproduced in Fig. 1, which also shows the individual course of each patient. Finally, the variations in extracellular volume, standard bicarbonate and serum calcium did not correlate to the changes in the TmP/GFR index.

DISCUSSION

The influence of vitamin D on the renal handling of phosphate has been a matter of controversy (17, 22). The availability of the biologically active $1\alpha,25(OH)_2D_3$ or the more convenient 1α OH D_3 has recently made it possible to study the renal effects of these compounds. In the present investigation, long term administration of 1α OH D_3 increased the TmP/GFR index and serum calcium significantly and gave a significant decline in i PTH. Furthermore, the linear correlation persisted between TmP/GFR and i PTH after the treatment with 1α OH D_3, while serum calcium, standard bicarbonate and the extracellular volume remained uncorrelated to the TmP/GFR index. It is therefore concluded that 1α OH D_3 increases the tubular reabsorption of phosphate and reduces i PTH in a parallel manner. This suggests that the 1α OH D_3 induced changes in the renal handling of phosphate may be explained solely by the parallel suppression of i PTH.

The suppression of PTH may be either a direct effect of 1α OH D_3 (or $1\alpha\ 25(OH)_2\ D_3$) on the parathyroid glands, or an indirect effect through an increase in ionized calcium, or any combination of these.

An increase in total serum calcium was seen after 1α OH D_3 treatment. As the calcium ion may also have a direct renal effect (3, 13), the present findings do not rule out the possibility that the increase in serum calcium, in addition to the suppression of PTH, may have contributed to the increase of the TmP/GFR index after 1α OH D_3 treatment. It has further been suggested (9, 22) that vitamin D exerts a direct action on the renal tubular transport of phosphate. This cannot be ruled out by the present study either. Clarification of a direct renal effect of calcium and the biologically active D vitamins presupposes studies where the PTH concentration remains constant during the administration of vitamin D. Therefore studies in totally parathyroidectomized patients may serve to clarify the possible direct action of ionized calcium and the biologically active D vitamins on the tubular handling of phosphate.

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Clinical and Laboratory Findings in Subjects with Hypercalcaemia

A Study Including Cases with Primary Hyperparathyroidism Detected in a Health Screening

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ABSTRACT Primary hyperparathyroidism (PHPT) was the most likely diagnosis in 68 non thiazide treated patients with hypercalcaemia detected in a health screening The group comprised 55 females and 13 males, with a mean age of 55 0±0 7 (S E M) years On a pair basis these patients (the observation group) were compared with a series of 68 age- and sex matched normocalcaemic subjects (the control group) selected from the health screening register Renal calculi and reduced creatinine clearance were encountered less frequently in the observation group than in many reports of hospitalized patients with PHPT Compared with the control group, the observation group comprised a greater number of subjects with renal calculi (usually multiple and bilateral) constipation mental depression and reduced creatinine clearance The ECG Q–T interval was shorter in the observation group than in the control group No differences were found with respect to the occurrence of gastritis and/or peptic ulcer, polydipsia polyuria and general muscle weakness On the basis of this and a previous study it was concluded that at least 3‰ of the 15903 subjects participating in the health screening suffered from "asymptomatic" hypercalcaemia and most probably from asymptomatic' PHPT

Health screenings provide an opportunity of detecting asymptomatic disorders at an early stage An example of such a disorder is primary hyperparathyroidism (PHPT) which may be present without any clinical symptoms or laboratory abnormalities apart from hypercalcaemia (HC) (9) PHPT is also known to be associated with various symptoms that make the patient aware of his disease The manifestations most frequently encountered are e g renal calculi gastritis and/or peptic ulcer constipation polydipsia polyuria muscle weakness and mental depression These findings have been reported in several publications (5 9 16 21 25 and others) mainly covering patients admitted to hospitals or out patient departments

In a recent paper we reported on the prevalence of HC detected in a health screening (2) The aim of the present paper was to characterize clinical manifestations in subjects from this health screening with repeated determinations of HC The findings were compared with those in a control group matched by age and sex

MATERIAL

A health examination is offered regularly to employees of the Stockholm City and County Council Employees aged 50 or over are examined every two years and those under 50 every five years At these examinations performed at different personnel surgeries (special clinics for health controls of the employees) the physician records the histories and examines the persons according to a standardized procedure Specimens of blood and urine (of non fasting subjects) are collected for analysis A routine ECG examination is performed

The observation group Subjects health-examined from July 1971 to July 1973 with a serum calcium level ≥11 1 mg/100 ml (Auto-Chemist AGA AB Stockholm) were reinvestigated and 95 (6‰) were found to have HC after repeated determinations (verified HC VHC) Twenty seven subjects treated with thiazide type drugs and/or suffering from diseases other than PHPT that may be associated with HC were excluded The remaining 68 subjects formed the observation group 56 of them were reinvestigated at Serafimerlasarettet where VHC is defined as a serum calcium level ≥2 64 mmol/l in at least two out of three consecutive determinations (atomic absorption spectrophotometry) The other 12 subjects were reinvestigated and subsequently operated upon in other hospitals (VHC defined according to local criteria)

Parathyroid adenomas were revealed in all these cases Causes of HC other than PHPT were excluded in a follow up as far as possible (2) Of the 68 subjects in the observation group 44 have hitherto been subjected to explorative neck surgery and parathyroid adenomas were found in all but two cases In all patients in whom adenomas were found the elevated serum calcium values normalized after surgery The age range in the observation group was 35–63 years (55 0±0 7 mean ±S E M) 55 were females (age 55 7±0 6 years) and 13 males (age 52.5±2 4 years)

The control group A control for each subject in the observation group was selected from the health screening register In accordance with the principle of selecting matched paired samples (15) each control had to be of the same sex and born in the same year and month as the corresponding subject in the observation group One of several possible control candidates was chosen at random and accepted providing he/she had been examined at the same personnel surgery in the same year and month as the corresponding subject in the observation group The 68 subjects selected in this way constituted the control group all invited controls participated in the study

Procedures

All subjects in both groups visited the hospital where their histories were scrutinized further by one and the same investigator according to a standardized questionnaire (preoperative histories were obtained in those 12 whose necks were explored before the present follow up)

Histories of heart failure myocardial infarction sarcoidosis diabetes mellitus hypothyroidism hyperthyroidism alcoholism schizophrenia pancreatitis and all disorders treated surgically were verified from hospital records Angina pectoris was defined according to Rose (27) Reports of hypertension urinary tract infection and gastritis and/or peptic ulcer had been diagnosed and treated by physicians in all cases The diagnosis of renal calculi was based on X rays

Constipation polydipsia polyuria general muscle weakness and mental depression (defined as an affective dejection in which the patient was unhappy pessimistic and self-depreciating) were recorded when the subjects had spontaneously noted these symptoms during a substantial part of the two years prior to the follow up Information concerning periods away from work because of sickness was obtained from the Social Security Office Data on blood and urine analyses were obtained from the health examination records Creatinine clearance was determined in duplicate (single determination in patients subjected to explorative neck surgery prior to follow up) and an X ray of the urinary tract was taken Relative body weight was calculated according to the formula

$$\frac{\text{body weight (kg)}}{\text{body height (cm)} - 100} \times 100$$

METHODS

Chemical analyses were performed according to routine methods including multichannel blood tests (AutoChemist) Routine ECGs with subjects in a recumbent position including leads I II III aVR aVL aVF and six chest leads CR_1–CR_6 and CR_7 were recorded using an ink jet recorder (Mingograph Elema Schonander AB Stockholm) at a paper speed of 50 mm/sec To estimate the corrected Q–T interval (Q–T_c) a nomogram (10) was used to calculate the Q–T interval corresponding to a heart rate of 60 beats/min

Statistics

Student s *t* test for paired samples was used in comparisons of quantitative variables Fourfold tables were constructed on a pair basis for differences in qualitative variables and the hypothesis equal or higher frequencies in the control group was tested against the alternative hypothesis higher frequencies in the observation group" using the binomial test (1) Data are presented as mean ±S E M

RESULTS

Histories and clinical findings

Single subjects in the observation and control groups had a history of myocardial infarction angina pectoris heart failure diabetes mellitus hypo- and/or hyperthyroidism The latter five disorders were all under adequate management with drug therapy Three subjects in the control group had been treated for sarcoidosis pancreatitis and schizophrenia respectively Histories of arthralgia in any form were reported by 44 subjects in the observation group and by 42 in the control group Partial gastrectomy because of duodenal or gastric ulcer had been performed in four subjects in the observation group and in two in the control group Two from each group had undergone cholecystectomy Two women in the control group had been operated upon for carcinoma of the uterine cervix and mammary gland respectively two women in the observation group and one in the control group for ovarian cysts

The physical examinations were in most cases unrevealing Mean values of BP recordings from the latest health screening were 156 0±2 1 (systolic) and 90 8±1 0 (diastolic) in the observation group and 137 4±1 6 (systolic) and 83 2±0 9 (diastolic) in the control group Details about the results of blood pressure registrations will be presented in a forthcoming paper (3) The occurrence of clinical findings considered to be of particular interest in relation to HC and PHPT is shown in Table I The two groups differed with regard to renal calculi (in most cases multiple and bilateral Table II) constipation and mental depression which were

Table I *Number of subjects with findings and disorders of particular relevance to hypercalcaemia and primary hyperparathyroidism*

	Observation group	Control group
Previous and current		
Renal calculi	16***	2
Urinary tract infection	15	12
Gastritis and/or peptic ulcer	16	14
Hypertension[a]	5	5
Observed in the past two years		
Renal calculi	9**	2
Urinary tract infection	5	6
Gastritis and/or peptic ulcer	4	4
Constipation	14**	4
Polydipsia	7	7
Polyuria	10	8
General muscle weakness	2	1
Mental depression	14**	3
At least one period of ≥7 days away from work because of sickness	6	5
Drug consumption		
Sedatives hypnotics	7	9
Antihypertensives	5	5
Laxatives	6	7

[a] Two patients in the control group used bendroflumethiazide the treatment otherwise consisted of methyldopa propranolol and hydralazine in both groups

The differences are not significant unless otherwise indicated

** Significantly different from controls ($p<0.01$)

*** Significantly different from controls ($p<0.001$) When two male pairs were excluded the level of significance was $p<0.01$

more common in the observation group A diagnosis of renal calculi in the 12 years prior to the follow up was found in 16 (24%) patients in this group (14 women and 2 men) Nine (13%) of them had this diagnosis confirmed in the two years prior to follow up eight (12%) of whom with current findings including one case with calcified deposits in parenchymal tissue in addition to calculi Of the 16 patients with renal calculi seven had had episodes of renal colic for which two of them had received surgery both with recurrent episodes Six of the 16 patients had been treated for urinary tract infections All the patients with histories of renal calculi in the observation group had surgically verified parathyroid adenomas Previous and current findings of renal calculi were found in two controls (3%)

In the observation group 14 subjects (21%) complained of constipation against four (6%) in the control group The corresponding figures for mental depression were 14 (21%) and three (4%) respectively The two groups did not differ with respect to occurrence of urinary tract infection gastritis and/or peptic ulcer hypertension polydipsia polyuria general muscle weakness drug consumption and periods out of work because of sickness A total of 48 subjects in the observation group and 60 in the control group had no histories of renal calculi constipation or mental depression ($p<0.01$)

Relative body weight averaged 104±2 and 102±2% in the former and latter group respectively (N S)

Laboratory findings

The mean value for serum calcium at the health screening was 11 5±0 1 mg/100 ml (range (11 1–13 0) in the observation group and 10 0±0 1 mg/100 ml (range 9 0–10 9) in the control group ($p<0.001$)

Hb ESR urinary glucose urinary protein and Auto-Chemist results for potassium sodium chloride phosphate urea nitrogen creatinine glucose total protein albumin haptoglobin bilirubin ASAT ALAT LDH ALP zinc sulphate reaction cholesterol iron TIBC and uric acid failed to display any significant differences between the observation and control group Mean values for total protein and albumin in the former group amounted to 7 4±0 1 and 4 1±0 1 g/100 ml and to 7 3±0 1 and 4 0±0 1 g/100 ml in the latter group No protein or glucose could be detected in the urine of any members of the two groups Values for creatinine clearance less than 85 ml/min were recorded in nine subjects from the observation group averaging 61±4 ml/min (range 39–76) This parameter was within normal limits in all members of the control group ($p<0.01$) Excluding the nine patients mentioned above and their matched controls creatinine clearance averaged 99±5 ml/min in the observation group and 102±4 ml/min in the control group (N S) Recording of parathormone (PTH) was not a part of the health control screening during the actual period but has been presented in a previous study (2)

The mean value for the Q–T interval was 0 34 sec (S D 0 04) and 0 41 sec (S D 0 06) for the observation and control group respectively ($p<0.001$) These values did not change when subjects associated with factors possibly capable of affecting

Table II *Renal calculi in the observation group All findings were located within calyces and pelves*

	Solitary	Multiple	Total
Previous and current			
Unilateral	4	3	7
Bilateral	1	8	9
Current			
Unilateral	2	1	3
Bilateral	–	5	5

the Q–T interval were excluded (10 in the observation group and 8 in the control group with corresponding pair mates) Such factors included digitalis treatment heart failure history of a myocardial infarction and hypertension (12)

DISCUSSION

The subjects in the present observation group did not differ from their matched paired controls with regard to age sex overweight past and current histories of most medical surgical and psychiatric diseases drug consumption and in the outcome of most laboratory tests performed However the values for their mean serum calcium level differed significantly In accordance with this finding the Q–T_c interval in ECG recordings was shorter in observation group subjects than in controls (25) So judging by findings in a previous investigation (2) most hypercalcaemic subjects included in the present study were apparently suffering from PHPT Thus 42 of the 44 subjects hitherto operated upon proved to have parathyroid adenomas

The HC of the subjects in the observation group was detected accidentally i e in a health screening In view of this circumstance it was assumed that the disease of the present patients was either detected in an early stage or that manifestations were less pronounced than those reported in many other publications comprising hospitalized patients with HC and PHPT Inclusion of the normocalcaemic control group studied under identical conditions made it possible to evaluate findings associated with HC and PHPT in patients not yet admitted to hospital

Even though most subjects in both groups considered themselves to be in reasonably good health their histories disclosed a number of symptoms and disorders Owing to the diffuse character of some of these manifestations their evaluation is rather rough When this circumstance and the errors possibly due to bias in evaluation of the histories were disregarded it was concluded that the observation group differed from the control group

Table III *Occurrence of manifestations (%) characteristic of hypercalcaemia and primary hyperparathyroidism in some studies on hospitalized patients compared with the present findings*

Reference	No of subj	Renal calculi	Constipation	Mental depression
McGeown & Morrison (17)	53	91	~100	
Hellström & Ivemark (7)	138	67		
Lemann & Donatelli (12)	46	29	37	
Cope (4)	343	57		
Winter & McQuarrie (29)	24	88	67	
Purnell et al (24)	171	51		
Johansson et al (8)	208	54		
Laumer (11)	38	55	16	
Lievre et al (14)	100	57		12
Mosekilde et al (18)	36	53	33	11
Romanus et al (26)	274	73		
Mallette et al (16)	57	32	32	
Werner et al (28)	129	53		
The present study				
Observation group	68			
Previous and current		24		
In the past two years		13	21	21
Control group	68			
Previous and current		3		
In the past two years		3	6	4

bo h w th regard to the occurrence of renal calculi and mpaired renal funct on (decreased creat nine clearance) and with regard to constipation and mental depress on This d fference was not reflected n the use of sedatives hypnot cs or laxa ti es A number of other symptoms commonly assoc ated with HC and PHPT (e g gastritis and/or pept c ulcer hypertens on polydipsia polyuria and general muscle weakness) were observed to the same extent n both groups

Al ogether 48 subjects in the observat on group (71%) d splayed asymptomatic HC when renal calculi const pat on and mental depress on were employed as ind cations of symptomatic HC Our pr v ous f nd ngs (2) d sclosed that 95 of the 15 903 part c pants (6 ‰) in the health screen ng project ha e VHC and most of these probably have PHPT So approximately 3 ‰ of the whole health screened populat on may have asymptomatic PHPT A total of 20 subjects in the observation group (29%) and 8 subjects in the control group (1 %) currently had one or more of the three symptoms ment oned above This relatively small difference clearly shows once again that HC and PHPT d splay rather unspecific cl n cal p ctures

Twenty four per cent of the subjects in the observat on group and 3% in the control group had h s ones of past or current nephrol thiasis The s ones were generally mult ple and bilateral and as oc ated with impa red renal funct on in about half of the cases Thus all but one of the nine subjects w h reduced creatin ne clearance had or have had renal calcul Mult ple and bilateral renal con crements often n combination with renal damage are reported as one of the most common compl ca tions in PHPT (7 9 20 25) The occurrence varies grea ly (Table III) but is generally much more frequent than in the present study This discrepancy may in part reflect the fact that the PHPT of the present pat ents was detected at an early stage of the d sease However a long PHPT duration need not necessarily be l nked to a wider or more compli ca ed spectrum of man festations (9) It is notable tha some of the present pat ents had suffered from renal calcul up to 12 years before the study

Const pat on and mental depress on were each observed n 21% of the subjects in the observat on group These f gures are in fa rly good agreement w th those found in several other publ cat ons (Table III) The registrat on of mental depress on as the only s gn of disturbed mental funct on may be a s mpl f cat on since PHPT is reported to be assoc ated with a var ety of ment l disturban e (6 22) However halluc nosi or other man fe a t ons characterist c enough to perm t ad q a el object ve registr t on were not ob erved n th present pat ents The f nd ng n som e rl er n vest gations that PHPT is assoc ated w th an n creased occurrence of gastrit s and pept ul e has not been conf rmed e ther in a numb subsequent reports (25) or n the pre ent stud However the observat on that gastr t nd pep ulcer in pat ents w ith PHPT may n pro follo n parathyro dectomy (25) suggests that PHPT und certa n cond t ons may be of som mp t n e n th s respect

Med cal screening n lud ng blood analy e the only way to detect asymptomatic HC When th derangement has been found however the phys cian must determine whether the pat ent suffers from PHPT and furthermore whether uncomp cated PHPT requ res surgery Neither of th decis ons may be easy First of al the levels n pat ents w th PHPT may fluctu s derably even normocalcaem c cases h t reported (17 19) Second no criter a hav e be identified making it poss ble to po nt out the tients whose disease w ll ult mately be me p gress ve In a prospective study Purn ll e found that the d sease in 20% of pat ents h b chem cal hyperparathyroid sm d spla ed p gressivity demand ng surg cal intervent n w tr five years of observat on Further stud es of th type are urgently needed

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Menopausal Age of Females with Hypercalcaemia

A St dy Includ ng Cases th Primary Hyperparathyro d sm Detected in a Health Screen ng

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ABSTRACT The mean age at natural menopause of 3-8 women participating in a health screening con ducted by the Stockholm City and County Council was 50 2 years The corresponding age encountered for a subgroup of 49 women with hypercalcaem a (ery probably due to primary hyperparathyro dism) conf rmed n repeated determinations was 45 7+0 4 (mean S E M) and signif cantly lower than that (50 1+0 4 years) of a normocalcaemic age matched control group ($p<0.001$) The health screening was performed about eight years after the women s menopause The observation and control groups showed a significant d fference with regard to the serum cal cium level but no difference was found with respect to marital parital or socio-economic status or dis orders that may result in early or late menopause

Primary hyperparathyro d sm (PHPT) may occur at any age It is rare in ch ldren and less common in men than n women who represent about 60-70% of reported cases The predominance of fe males over males is greater among persons aged 50 years or more (21) This is also llustrated by a report from the Cancer Incidence in Sweden (National Board of Health Stockholm) (2) (F g 1) This observat on suggests that the development of PHPT s to some extent related to the decline in ovarian function of the postmenopausal women However only ins gn f cant direct ev dence has hitherto been found showing that the female sex hormones exert an important effect upon parathy roid funct on Furthermore it is difficult to tell when PHPT init ally develops in a postmenopausal woman Symptoms of PHPT may appear several years prior to the d agnos s (14)

Al hough the evidence is partly contradictory several factors such as familial predisposition marital parital and soc o-econom c status age at menarche abnormal body we ght and the presence of various d sorders have been assoc ated with an early or late onset of menopause (6 7 8 10 11 12 13 15 16 17 19)

Clin cal man festat ons and laboratory find ngs subjects w th hypercalcaemia (HC) and PH detected in a health screening have been de nb prev ously (3 4 5) Summariz ng the h storie the female patients it was found that they becan menopausal at an earlier age than normocal aem age matched controls This f nding and its po b relationsh p to various factors known to influen e the development of the cl macterium are the sub jects of the current report

MATERIAL

A total of 135 women w th a s ngle recorded serum cal c um value ≥11 1 mg/100 ml (Auto-Chemist) found n a health screening were nv ted to take part n a follow-up examinat on E ghty of these women were found to have HC conf rmed n repeated determinat ons (verif ed HC VHC) Twelve were reexamined at other hosp tals where VHC was def ned by local cri eria The other 68 women examined at Serafimerlasarettet displayed a calc um level ≥2 64 mmol/l n at least two of three consecutive serum samples (atom c absorpt on spectrophotometry) At the follow up 25 of the 80 females were found to have pre v ously non-d agnosed diseases (hyperthyroidism hypo thyro d sm sarco dos s hypernephroma mammary car c noma) or were be ng treated with thiazides or thiazide like drugs (clopamide chlorthalidone) which n them selves may induce HC Four other women had not reached the r cl macteric period One had undergone an oophorectomy for ovarian cysts while st ll at a reproduc tive age and a sixth pat ent had not experienced any menstrual bleed ng following a cure tage performed when she was 35 years old These 31 women were excluded from the study The remaining 49 females with a natural

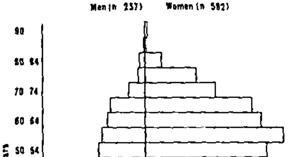


Fig 1 Patients (n=819) operated upon in Sweden because of parathyroid adenomas or carcinoma in 1959-1970 according to the Cancer Incidence in Sweden (National Board of Health Stockholm)

menopause referred to as the *observation group* were very probably suffering from PHPT (3 5) Twenty seven of these women have hitherto undergone explorative neck surgery As for the criteria for surgical intervention these will be dealt with in a forthcoming paper No selection took place except for single cases with extreme serum calcium elevation or renal failure Parathyroid adenomas were found in all cases and serum calcium decreased after surgery

The *control group* was selected among women participating in the health screening according to the principles of matched paired samples (18) They were born in the same year and month as their pair mates in the observation group Furthermore each pair of women had been examined in the same year and month at the same personnel surgery All controls invited agreed to participate in the study and all were postmenopausal

The premenopausal histories were penetrated in both groups (3) A few (up to four) women in the observation and control groups had histories of myocardial infarction angina pectoris heart failure hypertension iron deficiency anaemia diabetes mellitus hypo and/or hyperthyroidism all under adequate management with drug therapy Three controls had been treated for sarcoidosis pancreatitis and schizophrenia respectively One control was alcoholic Histories of arthralgia in any form were reported by 40 females in the observation group and by 39 in the control group Ten cases with histories of urinary tract infection were found in each group Partial gastrectomy because of duodenal or gastric ulcer had been performed in four women in the observation group and in one control Ten women in either group had histories of gastritis and/or peptic ulcer Two from each group had undergone cholecystectomy One control had been operated upon because of mammary carcinoma

No intergroup differences were revealed with respect to the disorders above Further details of the health screening the selection of control group and the various laboratory analyses are presented in previous papers (3 4 5)

PROCEDURES AND METHODS

All participants in the observation and control groups visited the Out patient Clinic of Serafimerlasarettet and a detailed history was taken by one and the same investigator

The female participants in the health screening filled out a standardized questionnaire including a question about the age at which cessation of menstrual bleedings appeared Information with respect to age at natural menopause was obtained from these questionnaires The age of a woman at the time of her menopause was defined as her age on the birthday prior to her last menstrual bleeding Menstrual bleedings had in all cases been absent for at least 12 months

About 85% of the women in both groups had undergone gynaecological examinations regularly (usually once a year) Data concerning menopausal age in the records from these examinations were compared with those given by the women at the follow up In general the women s recollections agreed with those of the records When diverging the women admitted that the records were correct

Relative body weight was calculated according to the formula

$$\frac{\text{weight (kg)}}{\text{height (cm)} - 100} \times 100$$

Statistics

Student s t test for paired samples was used in comparisons of quantitative variables Fourfold tables were constructed on a pair basis for differences in qualitative variables and the hypothesis "equal or higher frequencies in the control group was tested against the alternative hypothesis higher frequencies in the observation group" with the binomial test (1) Data are presented as mean ±S E M

RESULTS

The single calcium determinations at the health screening averaged 11 4±0 1 mg/100 ml in the observation group and 10 0±0 1 mg/100 ml in the control group (p<0 001) In all instances the serum calcium level was higher in observation group subjects than in corresponding control women

Some data of importance for reproductive age are listed in Table I The women in the observation group and the control group did not differ with regard to the age at menarche The menopausal age

Table I *Some data of importance in reproductive life*

	Observation group	Control group
Age at menarche (y)	13 1±0 3	14 1±0 3
Age at menopause (y)	45 7±0 4***	50 1±0 4
Duration of reproductive period (y)	32 6±0 3 *	36 0±0 3
Never married (no)	3	4
Age at marriage (y)	23 6±0 3	24 1±0 3
Nulliparous females (no)	5	6
Pregnancies per female (no)	2	2
Females with more than 4 pregnancies (no)	3	3
Age at first pregnancy (y)	25 4±0 3	26 2±0 2
Age at last pregnancy (y)	37 1±0 3	38 2±0 3
Spontaneous abortion (no of females)	3	2
Induced abortion (no of females)	2	2
Treated with oral contraceptives for 1-4 years (no of females)	5	4

** Significant difference from the control group ($p<0\ 001$)

was significantly lower in the observation group (45 7±0 4 years) than in the control group (50 1±0 4 years) ($p<0\ 001$) The mean age at natural menopause in 7 328 female participants in the health screening was 50 2 years based on information from standardized questionnaires In altogether 40 matched pairs the menopausal age of the woman in the observation group was lower than her corresponding pair mate s in the control group The mean menopausal age of the 27 women in the observation group who hitherto had undergone explorative neck surgery was 45 9±0 3 years The corresponding figure for their controls was 50 2±0 4 years ($p<0\ 001$) According to the data presented the duration of the reproductive period of life (between menarche and menopause) was longer for the 49 controls (36 0±0 3 years) than for their pair mates in the observation group (32 6±0 3 years) This difference was not reflected in any difference in the number of pregnancies age at first and last pregnancy duration of lactation periods number of abortions or in use of oral contraceptives

Histories of gynaecological data failed to disclose any differences between the two groups (Table II) Menstrual bleeding generally displayed normal duration and frequency Six women in the observation group and five in the control group had been treated with analgetics for dysmenorrhoea The gynaecological examination performed at the health screening did not disclose any unexpected pelvic disorders displacements or deformities with the exception of the postoperative condition of some women (Table II)

As shown under Material there were no inter group differences with respect to premenopausal histories concerning medical surgical and psychic disorders There were no apparent differences between the groups in respect to nutritional statu The follow up was performed when the wom were 55 5±0 6 years old i e about eight ye after their menopausal onset This examinati revealed that 14 women had or had had renal ca culi in four subjects associated with an impaire renal function as evidenced by a moderate decre in creatinine clearance Altogether 14 women had noticed constipation and/or mental depression during a significant part of the two years preceding the follow up The presence of these various mani festations was not reflected in menopausal age (Table III) Relative body weight averaged 103±2 and 101±2% in the observation and control groups respectively Four cases in each group were >120% overweight (N S) Exclusion of these cases and their pair mates did not influence the significant

Table II *Premenopausal gynaecological histories*

	Observation group	Control group
Dysmenorrhoea	6	5
Salpingitis	3	4
Colpitis	6	5
Carcinoma of the uterine cervix	–	1
Ovarian cysts	1	1
Uterine prolapse[a]	2	2
Menorrhagia	5	4

[a] Operated upon

Table III *Previous and current manifestations possibly associated with primary hyperparathyroidism found at the follow up*

Four cases of renal calculi in the observation group had been observed during premenopausal stage manifestations were otherwise found to be postmenopausal in all females The observation cases differed from their controls in all comparisons ($p<0\ 01$)

	Observation group		Control group	
	No of women	Age at menopause (y)[a]	No of women	Age at menopause (y)[b]
Renal calculi	14	46 1±0 5	2	50 1
Constipation	14	45 2±0 4	4	49 9
Mental depression	14	45 6±0 4	3	50 0

[a] Mean ±S E M [b] Mean

difference in menopausal age between the two groups

Most women in both groups were born and had grown up in the Stockholm area There were no intergroup differences with regard either to education and occupation (manual and non manual) or to family income About half of the women in each group knew at which age their mothers had reached menarche and menopause No intergroup differences were observed

DISCUSSION

Retrospective studies may be rather inexact concerning a woman s menopausal age because of the difficulties in recalling the correct date of the cessation of menstruation Inconsistencies in the ... ons used by various authors also contribute ... the variability of results Considering these problems McKinlay et al (19) when summarizing the literature concluded that the average menopausal age in well nourished populations was fairly constantly about 50 years In keeping with this concept the age at menopause averaged 50 2 years in the total series of women participating in the present health screening and 50 1±0 4 years among the women in the control group A somewhat lower age (48 9 years) has been reported for another series of Swedish women (23) However compared with these figures the one obtained in the current observation group (45 7±0 4 years) is definitely lower and significantly different from that recorded in the control group The circumstance that about 85% of the women in the control as well as in the observation group were examined regularly (mostly once a year) by a gynaecologist at the time of the menopause, supports the validity of the present results

Although some women in both groups had histories of diseases such as heart failure angina pectoris hyper and hypothyroidism and diabetes mellitus most of them considered themselves to be in good health at the time of the follow up The diseases mentioned above were adequately controlled by drug therapy These disorders as well as those affecting the reproductive organs were about equally frequent in both groups However the women in the control and observation groups had significantly different serum calcium levels higher in the observation group On the basis of previous studies (3 5) it was concluded that HC in most cases probably was associated with PHPT In the absence of differences in factors such as marital parital and socio-economic status, it appears that the early onset of menopause in the women of the observation group may be primarily associated with the HC and/or other consequences of a deranged parathyroid function

Debilitating diseases, major nutritional disturbances and endocrinological diseases due to lesions of the pituitary gland the adrenals the pancreas and the thyroid may all contribute to the development of amenorrhoea The early onset of menopause in the women of the present observation group suggests that PHPT under certain circumstances may have a similar effect However it is impossible to determine on the basis of the present data whether PHPT or menopause occurred first An antagonism has been demonstrated between parathyroid hormone and oestrogens upon bone (22) The relation between menopause and a nega

tive calcium balance has been studied by e g Nordin (20) Administration of ethinyloestradiol to postmenopausal women with PHPT and HC has been shown to lower plasma and urinary calcium (9) Whether parathyroid hormone interferes with the effect of oestrogens or with the metabolism of other hormones regulating the menstrual cycle remains to be established

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Acetylator Phenotype in Patients with Hydralazine-induced Lupoid Syndrome

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ABSTRACT The acetylator phenotype has been determined (isoniazid half life) in 31 patients, 25 of them women, who had exhibited a lupus erythematosus like syndrome during treatment with hydralazine Twenty nine patients were slow acetylators, one was rapid (probably spontaneous SLE) and one uncertain Only two patients had been given more than 200 mg of hydralazine daily The mean duration of therapy was 32 months at the onset of symptoms These were not serious but rather long standing Our study confirms that patients who risk developing hydralazine lupus are slow acetylators especially females, treated with more than 100 mg daily Rapid acetylators seem to develop this side-effect rarely if at all

Hydralazine (Apresolin®) was the first drug reported to induce a syndrome similar to systemic lupus erythematosus (SLE) (20) An important pathway for the metabolism of hydralazine is acetylation which is under polymorphic genetic control (12 26) The enzyme involved also acetylates other drugs such as isoniazid (INH) (11) certain sulfonamides (12 29) and procaine amide (13 19 27) Slow acetylators are more prone to develop side effects on conventional doses of INH (7) phenelzine (10) sulphapyridine (5 29) and dapsone (8) Perry (25) found that nearly all patients who developed a lupoid syndrome during treatment with hydralazine were slow acetylators and that positive antinuclear factor (ANF) preceded the clinical symptoms By contrast Alarcón Segovia et al (2) and Evans et al (9) did not find a significant difference between slow and rapid acetylators in the incidence of positive ANF during long term treatment with INH Recent studies suggest that procaine amide may induce a lupoid syndrome not only in slow (20) but also in rapid acetylators (6 17)

In the light of these somewhat conflicting reports we have phenotyped patients in whom a lupus like syndrome had occurred during treatment with hydralazine A local compilation of hydralazine induced lupus (16) initiated our study which was enlarged to a whole country survey aided by the Swedish Adverse Drug Reaction Committee

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PATIENTS

The reports to the Swedish Adverse Drug Reaction Committee on hydralazine induced lupoid syndromes during 1968–74 were analyzed The reporting physicians were then contacted to obtain the clinical records It was possible to arrange for acetylator phenotyping in 31 patients through cooperation with 17 of the reporting colleagues

Age and sex distribution hydralazine dosage and duration of treatment

The patient material consisted of 6 men and 25 women with a mean age of 58 years All the men were under the age of 60 while the majority of the women were older than that (Table I) when symptoms of the lupoid syndrome first appeared

The dosage of hydralazine and the duration of therapy before the onset of symptoms are shown in Table II Only two patients were given more than 200 mg of hydralazine daily and only during part of the treatment period The mean duration of therapy at onset of sym

Table I *Age and sex distribution of 31 patients with SLE during hydralazine treatment*

Age at onset of symptoms (y)	Men	Women
40–49	3	5
50–59	3	6
60–69	0	10
70–79	0	4
Total	6	25

Table II *Dosage and duration of hydralazine therapy*

Hydralazine dose (mg/d)	Duration of therapy <6 mo	6–12 mo	1–2 y	>2 y	Total
<100	1	1	1	4	7
100–200	1	2	7	10	20
>200				2	2
Total	2	3	8	16	29*

* To be added one patient treated with <100 mg duration unknown one patient treated >2 years dosage unknown

toms was 32 months (range 3 mo – 8 y) Serum creatinine was normal in 8 elevated in 11 and not reported in 12 patients The patients with impaired kidney function had a hydralazine dosage and duration of therapy similar to those of the other patients

METHODS

Acetylator phenotyping was performed by giving a single oral dose of INH (10 mg/kg b wt) after fasting over night Only indispensable drugs none of which being acetylated were allowed during the day of the test and 24 h before it Venous blood samples were drawn 2 3½ 5 6½ and 8 h after the dose (in some patients only 4 samples) and heparinized plasma was sent and stored frozen for analysis within one week Plasma concentrations of INH were determined spectrophotometrically (23) The elimination rate constant (λ) was calculated by least squares regression analysis and the plasma half life ($T\frac{1}{2}$) derived from the equation $\ln 2/\lambda = T\frac{1}{2}$ Patients with $T\frac{1}{2}$ longer than 2 Γh were classified as slow acetylators (15)

RESULTS

General clinical findings

In 28 patients the lupoid syndrome started with joint symptoms i e pain swelling and redness over the joints of the fingers wrists elbows shoulders knees and ankles, in some patients only as migratory pains Only single cases exhibited other symptoms of SLE according to the classification of the American Rheumatism Association (4) (Table III) Twenty eight patients had positive ANF LE cells or both no serological investigations were performed in three patients Three patients had no joint symptoms but developed increasing fatigue elevation of the ESR to 100 mm/h or more anemia and increased γ globulins Two of these patients had ANF in titers of 1/100 and 1/2048 respectively The third patient had ANF in a titer of 1/25 dyspnea and marked bilateral pulmonary infiltrations

The treatment with hydralazine was continued

Table III *Symptoms and laboratory findings of SLE*

Positive findings	No of pats
Joint pains	28
Positive ANF LE cells or both	28
Joint pains and ANF/LE cells	24
Pleurisy or pericarditis	3
Leucopenia (<3 000/mm³)	3
Discoid lupus	1

for 1–12 months (mean 3) after the onset of symptoms Information on the clinical course following discontinuation of hydralazine was obtained for 27 of the 31 patients The follow up period was up to 6 years (mean 6 months) Twenty six patients improved subjectively during the months after the discontinuation of therapy but all did not recover within the observation period Consecutive chest X rays of the patient with bilateral pulmonary infiltrations showed marked improvement within ? months Only one patient did not improve (see below) Repeated ANF and LE cell tests were performed only in 13 patients In 10 patients a normalization was observed while the tests were unchanged in 3 patients followed for about 6 months

Acetylator phenotype

The distribution of the INH $T\frac{1}{2}$ is shown in Fig 1 Twenty nine patients were slow acetylators and one was rapid One patient had an irregular plas concentration curve making the classification uncertain with an approximate INH $T\frac{1}{2}$ of 2 3 h

CASE REPORTS

The rapid acetylator was the only patient who did no improve after cessation of hydralazine therapy She wa

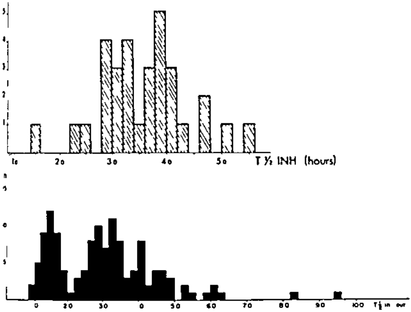


Fig 1 Distribution of isoniazid plasma half lives ($T\frac{1}{2}$) in 31 patients with SLE during hydralazine treatmen (top) and in 130 Swedish tuberculous patients (15) (bottom) $T\frac{1}{2}$ longer than 2 1 h indicates slow acetylation

a 49-year-old woman with an INH $T\frac{1}{2}$ of 1 5 h For some years she had had an unexplained elevated ESR She had been treated with 75 mg of hydralazine daily for hypertension during 1½ years when she developed exanthemas in the face and the scalp and pains in the right elbow A skin biopsy showed lupus erythematosus but no LE cells were found Hydralazine was discontinued and she has since been followed for 4 years During this period ANF became positive in titers varying between 1/10 and 1/25 and she had periodical symptoms from the skin and joints necessitating treatment with chloroquine

The patient with uncertain acetylator status was a 52 year-old woman treated with 100 mg of hydralazine daily for 1½ years She then developed pains and swelling of ankle and knee joints whereupon hydralazine was discontinued Initially LE cells were found in one of several samples ANF was positive in a titer of 1/8 and antibodies to Yersinia enterocolitica in a titer of 1/320 (complement fixation test) During her stay in hospital further LE cell tests were negative and no hematoxylin bodies or rosettes were found One and a half months after the onset of symptoms LE cells hematoxylin bodies and rosettes were strongly positive but the titer against Yersinia had decreased to 1/40 and the patient was free from symptoms One month later ANF and LE cells were negative

DISCUSSION

The clinical material reported in this study comprises 31 patients with symptoms and laboratory signs of SLE during treatment with hydralazine A causal relationship between the lupoid syndrome and therapy with hydralazine has been considered as probable in all 31 patients by the Swedish Adverse Drug Reaction Committee It should be noted that one of us (L H) has reported 10 of these cases more likely because of personal interest rather than geographical clustering

The symptomatology in this material was relatively meagre but similar to that reported by others e g Perry (25) According to Alarcón Segovia et al (3) hydralazine induced lupus syndrome cannot be distinguished from spontaneous SLE clinically serologically or pathologically

The daily dosage of hydralazine is low in this Swedish material compared with that of Perry (25) Two-thirds of the patients were treated with 100–200 mg and only two had received more than 200 mg Two patients were given less than 100 mg daily one during less than one year but she was also simultaneously treated with procaine amide The other had been on hydralazine therapy with a larger dosage 5 years earlier Large doses during prolonged periods are commonly considered to be necessary for the occurrence of the lupus syndrome (24) Thus Perry reported that 80% of his patients were treated with more than 400 mg daily On the other hand symptoms occurred in 15 of 48 patients on 200 mg daily or less (3) In the

present material the mean duration of therapy was 2½ years before the first symptoms occurred about the same as in other studies

The patients in this study had disturbing and rather long standing symptoms However no serious or fatal complications occurred and satisfactory clinical improvement was observed after the cessation of therapy The syndrome has previously been considered to be reversible but symptoms remaining for several years have been reported (1 25) especially when the treatment was continued after the appearance of symptoms (25)

Determinations of INH $T\frac{1}{2}$ in plasma were chosen for acetylator phenotyping because of previous experience with the method including the availability of Swedish reference material (15) The acetylator phenotyping indicated that 29 patients were slow acetylators one rapid and one uncertain This is significantly different ($p<0.01$) from the expected frequency of 68% slow acetylators (15) This is in accordance with previous results Thus Perry (25) reported that 24 of his 25 patients were slow acetylators as were all 11 patients tested by Hahn et al (14) The INH $T\frac{1}{2}$ were distributed approximately normally within the slow mode and extremely long half lives were not found (Fig 1) Although a control population of hypertensives has not been acetylator phenotyped it seems unlikely that hypertensive patients treated with hydralazine should differ from the general population with respect to acetylator status On the other hand a preponderance of slow acetylators has been reported in patients with spontaneous LE (22 28) The marked female preponderance and in this and earlier materials (25) remains unexplained

A positive correlation between plasma hydralazine concentrations and antihypertensive response has been established (18 30) The fact that predominantly slow acetylators develop hydralazine lupus suggests that the parent drug evokes the lesion If so however rapid acetylators treated with large doses should also experience this side effect As long as the detailed pharmacokinetics of hydralazine and its metabolites are not fully explored in this context it seems unjustified to assume that the higher plasma concentrations of unchanged hydralazine in slow acetylators are responsible for the lupus syndrome Perhaps drugs and endogenous primary amines which are substrates for acetylation have additive etiological roles in the development of the lupus syndrome

The present results confirm that acetylator phenotyping is useful for identifying patients who risk exhibiting hydralazine lupus These are slow acetylators especially females treated with more than 100 mg of hydralazine daily

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Immediate and Long-term Results of Emergency Aortic Valve Replacement in Acute Bacterial Endocarditis

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ABSTRACT A surgically treated material comprising 18 patients with heart failure from aortic insufficiency during acute endocarditis has been reviewed At the time of operation the mean duration of heart failure was 3 weeks and duration of endocarditis 9 weeks Blood culture was positive in half of the patients, 39% had predisposing valve disease, 14 (78%) had a preoperative heart catheterization The peroperatively measured regurgitation averaged 55% All 18 patients had an artificial valve implanted, and the mean observation time for 13 long term survivors was 3 1/3 years There were 3 postoperative and 2 late deaths A long term survival rate of 73% strongly supports early surgical treatment in patients with aortic insufficiency and heart failure during acute endocarditis

The most common causes of death following acute endocarditis before and during the early days of the antibiotic era were complications of general sepsis Nowadays the most frequent cause is uncontrollable cardiac insufficiency owing to destruction of the valves of the heart (5) In recent years surgical treatment has become a well established practice in such cases The results in the majority of materials are satisfactory having a total mortality rate of 25–40% (1 6 7) against nearly 100% in non surgically treated patients Aortic insufficiency is the most frequent sequela from valve destruction in patients with acute endocarditis

We have therefore carried out a survey of the 18 patients operated on in our department during 1968–74 for this condition All these patients had only the aortic valve replaced and surgical procedures were not performed on the other valves

MATERIAL

The material consists of 18 patients 16 men with a mean age of 41 2 years (range 15–65) and 2 women of 45 and 65 years The patients were referred for operation owing to severe and increasingly uncontrollable cardiac insufficiency during endocarditis All but two were on antibiotic therapy at the time of operation The duration of the acute endocarditis averaged 9 weeks (range 2–18) Cardiac insufficiency had on an average been diagnosed for 3 weeks prior to operation

Disposition to endocarditis in the form of earlier valve disease was found in 7 (39%) of the patients Three had aortic insufficiency two aortic stenosis and two a bicuspid valve Five (28%) of all the patients had previously had sepsis like conditions and possibly endocarditis of these only 2 were predisposed to valve disease the one with an aortic insufficiency and the other with an aortic stenosis

Blood culture was carried out several times in each patient but bacterial growth was found in only half of the cases Streptococcus viridans was found in 4 patients Staphylococcus aureus in 2 Pneumococci in 1 and Staphylococcus albus in 2 patients In the latter 2 patients a possibility of contamination is always present All of the patients had been treated with antibiotics after admission to hospital but first after the blood culture

Preoperative left sided heart catheterization (Table I) was carried out in 14 (78%) of the patients The 4 non-catheterized patients were among those 6 operated on before 1970 All patients operated on after 1970 were catheterized preoperatively No embolic complications have been observed in connection with this examination On the other hand 2 patients had had haematuria during the period of sepsis prior to operation possibly indicating renal embolus No other embolic manifestations have been observed

The relative heart volume averaged in 11 patie preoperatively 630±110 ml

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Table I *Preoperative circulatory parameters*
HR=heart rate LVEDP=left ventricular end-diastolic pressure

	HR	Aortic pressure (mmHg) Systolic	Aortic pressure (mmHg) Diastolic	LVEDP (mmHg) Before angiography	LVEDP (mmHg) After angiography
Mean	97	127	45	22	25
S D	15	21	19	11	14
n	18	18		14	9

The degree of aortic insufficiency on angiography was evaluated as grade III or more The insufficiency of the individual stroke volume was measured *peroperatively* by electromagnetic flowmeter (Nycotron Oslo) applied to the root of the aorta prior to bypass The degree of insufficiency was thus measured in 14 of the patients and averaged 55% (S D 15) The highest percentage of regurgitation was 85

Operative procedure

The Rygg–Kyvsgård heart–lung machine was used and the extracorporeal circulation had a mean duration of 85 min (range 52–124) and the aorta was clamped 68 min (range 40–112)

Perforation or partial destruction of one or more cusps was found at operation in 11 cases (61%) often with granulation tissue or fresh excrescences In 7 cases the cusps were stiff fibrous or partly calcified a few with granulation tissue

Different types of valves have been implanted the Starr–Edwards valve prior to 1971 in 7 cases later th pivoting disc valves the Bjork–Shiley prosthesis in 5 cases and the Lillehei–Kaster prosthesis in 6

Apart from a more pronounced tendency to tachycardia the postoperative course and the circulatory condition did not differ from those of other patients selectively operated on for valve replacement

RESULTS

Three (17%) of the 18 patients died immediately after operation or in the early postoperative phase Two patients died later 2 and 18 months after oper

Table II *Preoperative data and postoperative course for 5 non survivors (3 early and 2 late deaths)*
HR=heart rate BP s/d=systolic and diastolic blood pressure LVEDP=left ventricular end-diastolic pressure

Case no	Sex	Age (y)	HR	BP s/d (mmHg)	LVEDP (mmHg)	Duration of endocarditis (weeks)	Symptoms of cardiac insufficiency (weeks)
ly deaths							
	♂	52	90	110/65	18	12	8
2	♂	27	120	105/40		6	4
3	♂	41	90	120/50	45	3	2
Late deaths							
4	♀	45	95	120/50	25	6	3
5	♂	44	100	110/55		12	8

Aortic insufficiency measured peroperatively in cases 3 and 5 to 70% and 60% respectively Preoperative catheterizat not performed in cases 2 and 5

ation which gives a total mortality of 28% (Table II)

Three patients had to be reoperated on within the first 24 hours owing to heart tamponade In one patient a left sided hemiplegia developed post operatively but disappeared rapidly The patient is healthy and employed 7 years after operation Permanent arrhythmias developed in two patients One of them acquired a 1st degree AV block which required no treatment the other had a right bundle branch block She died (case 4 Table II) and the post mortem examination revealed a focus of infection in the atrioventricular septum

A slight paravalvular leakage was found in one patient who had a Bjork–Shiley valve no 27 implanted at the age of 30 He is fully employed 2½ years after the operation

Microembolic episodes have been observed in 2 patients One is patient 5 in Table II The other with a Starr–Edwards valve no 10 has had attacks of faintings and amnesia of short duration without permanent cerebral damage

One of the patients has recently been reoperated on for mitral valve insufficiency (after the period of the survey) He had a Starr–Edwards valve no 10 implanted during endocarditis with an aortic insufficiency of 70% 6 years earlier at the age of 38 Vegetations and perforation of a cusp were found during the primary operation Postoperatively he had prolonged fever with growth of Staphylococcus aureus in blood culture and was treated with antibiotics for 2½ months Thereafter his condition was satisfactory and he was fully employed as a clerk with no cardiac complaints Recently he was admitted to our hospital with severe heart failure due to mitral insufficiency The last operation showed perforation of the anterior mitral leaflet and paravalvular leakage at the aortic prosthesis of 35% The leakage was sutured and a Bjork–Shiley valve no 31 inserted in mitral position

Late results

The observation period for the 13 survivors averages 3 years and 4 months (range 8 mo –7 y) Eight patients are back in their previous occupation or carrying out work of corresponding severity Two patients have lighter work but are fully employed while 3 have not resumed work but can presumably carry out light work The latter 3 patients all men are among the oldest in the material and

Type of bacteria	Type of valve implanted	Duration of ECC/aortic clamping (min)	Course
Staphylococcus aureus	Starr–Edwards 10	68/57	Died 1 hour postoperatively Irreversible ventricular fibrillation
"	Starr–Edwards 10	77/62	Died immediately after operation Ventricular pump failure Insufficient coronary perfusion for 10 min during operation
Staphylococcus albus	Lillehei–Kaster 18	105/86	Ventricular fibrillation on 3 4 5 and 8 days after operation Irreversible fibrillation on 10th day Post mortem diagnosis myocardial infarction
"	Lillehei–Kaster 18	80/60	Permanent RBBB postoperatively Died 2 months after operation Acute sepsis duration 24 h Post mortem diagnosis abscess in atrioventricular septum
Streptococcus viridans	Starr–Edwards 10	75/65	Hospitalized several times after operation haemolytic anaemia possible mitral insufficiency and microembolus Died 18 months postoperatively after acute cerebral insult with a large haematoma in the right parietal lobe

were at the time of the operation 49 63 and 65 years of age

Only 2 of the 13 survivors have been admitted to hospital after the primary operation owing to heart disease or sequelae after operation Both have been described the one who was reoperated on and the other who had microembolus

DISCUSSION

The present material of patients with aortic insufficiency during the course of an acute endocarditis does not differ from corresponding groups in other surveys (1 3 8) The typical indication for rapid operation is progressive circulatory insufficiency and the short periods of diagnosed endocarditis and cardiac insufficiency of 9 and 3 weeks respectively show the acute and severe course of the disease

All ten patients with affection of the aortic valves in the material presented by Crosby et al (3) had a predisposing valvular disease whereas only $^{3}/_{5}$ of our patients had had earlier valvular disease *when the two cases of bicuspid aortic valves are* included On the contrary only one of ten of the patients of Wise et al (8) had predisposing valvular disease when cases of bicuspid valves are excluded

The frequency of positive blood cultures varies considerably in the different materials and a frequency of 50% as in the present series is relatively modest compared with the finding of Black et al (1) that the result was negative in only 13% of their surgical material The reason for this is probably that the majority of our patients have been treated at home with an antibiotic prior to admission to hospital In an earlier Scandinavian material (2) growth was found in 5 of 7 patients *with aortic regurgitation during acute endocarditis*

Preoperative heart catheterization was carried out in only a few cases in many other materials in collective materials only in approximately 12% (1) *In the present material 78%* of the patients were subjected to heart catheterization preoperatively and there have been no complications in connection with the examination We find it reasonable to obtain both catheterization and angiographic data in order to decide whether or not more than *one* valve is involved Even though the material includes patients who were seriously ill the examination has been carried out and has often been planned and performed immediately prior to operation

The haemodynamic data emphasize the acute development of the aortic insufficiency as the systolic pressure has not yet become increased at the same time as the pulse amplitude is relatively low The end-diastolic pressure in the left ventricle is considerably increased and in quite a number *equal to the diastolic pressure* and some dilatation of the heart has occurred with an average relative volume of 630 ml Thus these data show the uncompensated heart s reaction to aortic insufficiency (8)

Preoperative measurement of the degree of insufficiency has apparently not been reported in previous surveys but the average of 55% regurgitation found in this material clearly shows the necessity of a corrective operation in these patients

The operative technique and the use of different types of valves do not seem to have any influence on the postoperative course It must however be stressed that both our patients who developed symptoms of microemboli had a Starr-Edwards *valve implanted All the patients were treated with* anticoagulants irrespective of the type of valve

A more thorough evaluation of the pre and peroperative course and the preoperative haemodynamic data in the three early deaths does not reveal any important conditions which would suggest that these patients belonged to a higher risk group (Table II) The two late deaths can both have been caused by the primary disease and the postoperative conditions In the first case an abscess in the atrioventricular septum could probably be the cause of both the permanent bundle branch block and the recurrence in the fatal sepsis In the second case the apoplexy may have been caused by a brain embolus

The total mortality of 28% is satisfactory considering the life threatening condition preoperatively It is also satisfactory that among the 13 survivors only 3 were unable to work The fact that *these patients belong to the oldest in the material* makes their handicap more explainable

In the material of Griffin et al (4) consisting of unoperated patients with endocarditis and aortic insufficiency 7 of 8 patients with *cardiac insufficiency* died within 1–2 weeks and 7 of 11 with only slight cardiac insufficiency within 6 weeks

The fact that 10 of our 13 long term survivors were able to carry on a normal life and that the

remaining 3 had a satisfactory life with a mean observation time for the whole material of $3^1/_3$ years is very encouraging. This strongly supports the argument for surgical treatment of patients with aortic insufficiency during the course of acute endocarditis.

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Problems Encountered in Long-term Treatment with Anticoagulants

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ABSTRACT The course of long term anticoagulant therapy in 114 out patients has been evaluated over a three-month period The evaluation was based on the registration of information from the clinical records and from two personal interviews with each patient The patients had attended the Anticoagulation Clinic for 141 weeks on the average The prothrombin complex activity (PP%) level was significantly lower in patients with bleeding episodes An apparently higher PP% level in patients with thromboembolic manifestations was not significant No bleeding was observed when the PP% was above 25 Warfarin resulted in relatively fewer bleeding episodes and more PP% values within the desired range (10–25) than phenprocoumon and bishydroxycoumarin The role of age, but not of moderate hypertension, as a risk factor was confirmed A probable adverse interaction of the anticoagulant and other drugs was found in 37 5% of the situations in which an interaction could be expected according to the literature

The value of long term treatment with vitamin K antagonists as well as the risk are influenced by a wide variety of factors 1) the physical condition intelligence and co-operation of the patient 2) the laboratory method of control 3) the anticoagulant used 4) other drugs and agents administered simultaneously and 5) the experience of the attending physicians with anticoagulant therapy This list illustrates that optimum treatment is difficult to achieve and indicates that further elucidation of practical problems emerging during the treatment may form the basis for improvements

Thus the purposes of the present investigation of patients on long term treatment with vitamin K antagonists were 1) To elucidate the relation between the observed effect on coagulation (PP%) and the frequency of bleeding episodes and thromboembolic manifestations 2) To compare three pharmacokinetically different coumarin drugs 3) To record possible interactions with other drugs

MATERIAL

All the patients on long term anticoagulant treatment who attended our Out patient Clinic during a three month period (Oct–Dec 1974) were included in the study The duration of treatment up to the beginning of the investigation averaged 141 weeks (range 4–608) At the end of the three month period the patients had been treated for a total of 16 103 weeks

Table I shows the diagnoses and the sex distribution of the 114 patients Most of them had undergone or were candidates for heart surgery or reconstructive vascular surgery They were referred to the clinic from the Departments of Thoracic Surgery and Cardiology and the physicians from these units were in charge of the treatment with anticoagulants The two departments receive patients from the entire western part of Denmark and the drug chosen (Table I) for anticoagulant therapy was that preferred in the hospital of their home town

Owren s P&P (prothrombin and proconvertin) technique (15) was used to measure the prothrombin complex activity (PP%) Determinations of the PP% were on the average carried out every 20th day but the interval between the analyses was extended up to 2 months if the PP% was stable and reduced to 2–4 days if it was unstable The patients were informed by letter about the PP% value the recommended dosage of coumarin drug and the date for the next control visit Efforts were made to keep the PP% between 10 and 25 of normal activity

METHODS

Each patient was interviewed by one of us (S H) at least twice during the three month period at an interval of more than two weeks All the information used in this study

Table I *Sex distribution of the patients and drug used in relation to primary disease*

	Women	Men	Phenprocoumon	Bishydroxy coumarin	Warfarin
Heart disease					
Arteriosclerotic	3	11	3	10	1
Rheumatic	21	8	11	15	3
Peripheral vascular disease					
Arterial	18	38	34	3	19
Venous	0	2	0	1	1
Mixed cases	5	8	5	4	4
Total	47	67	53	33	28

obtained directly from the patients and from the clinical records was recorded on a standard sheet The information from the clinical records was checked in the presence of the patient, with careful questioning about current and previous intake of any drugs (prescribed as well as others) The occurrence of an interaction with another drug was accepted 1) if the prothrombin level was more than 5% above or 2% below the therapeutic range at three successive visits or 2) if an adjustment in the coumarin dosage larger than 30% had been found necessary Furthermore the patients were questioned about their awareness of possible side-effects of coumarin anticoagulants and of interactions with hypnotics analgesics or anti-inflammatory agents

RESULTS

Table II shows the level of all the PP% values determined Most of the values are within the cho- therapeutic range No value above 25% was erved at the time of bleeding and no value below % was seen during a thromboembolic manifestation It should be noted however that we do not know the PP% in 54 9% of the bleeding episodes

During 37 5% of episodes with thromboembolic manifestations the PP% is registered as unknown because no single value could be considered representative during a protracted clinical development

Table III lists the nature of the 51 bleeding episodes No lethal haemorrhages were observed Obviously some of the episodes are minor bleedings and it is unlikely that all such events were noted in the clinical records In addition only 12% of the patients knew that there was a bleeding risk during treatment with the coumarin drug The average duration of treatment at the time of bleeding was 139 weeks (range 2–356) Two thirds of the bleeding episodes occurred in the age group 60–69 years whereas only one third of the patients were in this age group at the start of the treatment

Table IV shows the nature of the 24 thromboembolic episodes and their relation to the initial indication for anticoagulant treatment The episodes developed on the average 120 weeks (range 1–545) after the start of anticoagulant

Table II *Percentage distribution of the 5636 Owrens P&P tests (PP%) in 114 anticoagulated patients during 16 103 weeks of treatment and of the PP%s determined at the onset of observed bleeding and thromboembolic manifestation*

Level of PP%	All PP%s determined	34 patients with 51 bleeding episodes	17 patients with 24 thromboembolic manifestations
<10	6 9	31 4	0
10–25	64 2	13 7	25
>25	27 9	0	37 5
Unknown		54 9	37 5

Table III *Episodes of bleeding in 34 patients during 114 courses of anticoagulant treatment*

Ecchymoses purpura petechiae	23
Epistaxis	6
Haematuria	5
Subcutaneous haematoma	5
Postmenopausal bleeding menorrhagia	5
Bleeding from ear	2
Haemoptysis	2
Melaena	1
Eye complications	1
Haemarthrosis	1
Total	51

\ Episodes of thromboembolic manifestation (n=24) observed in 17 of the 114 patients on gulant treatment

duration of treatment at the time of the episode was 120 weeks

l episode	Arterio-sclerotic heart disease	Rehumatic heart disease	Peripheral arterial disease	Peripheral venous disease	Mixed cases	Total no of episodes
ial infarction	3	3	4			10
lerotic manifestation · limbs			6			6
ophlebitis	1				1	2
ieurysm			2		1	3
vascular disease		1				1
f the lung		1				1
arction		1				1
of episodes	4	6	12	0	2	24
of pats	14	29	56	2	13	114

Aortic aneurysms are listed as embolic episodes Most of the episodes of dial infarction occurred in patients with clerotic diseases Four of the instances of dial infarction were seen in 56 patients with ns of peripheral arterial disease The obser eriod for the 56 patients was 13–562 weeks = 159)

patients with thromboembolic manifesta d more values above 25% and fewer below an the total patient population (Fig 1) r neither this difference nor that between ulations with and without thromboembolic tations is statistically significant On the and patients with bleeding differed signifi cantly from the total patient population (χ^2=17 45 p<0 001)

The difference in the distribution of PP% values in groups of patients treated with different dr s was highly significant (χ^2=43 7 p<0 001) (Fig 2 Warfarin gave the lowest number of PP%s outside the therapeutic range Phenprocoumon led to more PP% values below 10% and patients treated with bishydroxycoumarin had more PP% values above 25 The average interval between the control visits was longest for warfarin treated patients (23 days) 19 days for those on phenprocoumon and 17½ days for those on bishydroxycoumarin

Table V allows a comparison between the fre quencies of bleeding episodes during treatment with



Percentage of PP% values above or below the range in all patients patients with throm manifestations and patients with bleeding

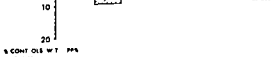


Fig 2 Percentage of PP% values above or below the therapeutic range during anticoagulation with three differ ent drugs

Table V Bleeding episodes during treatment with three different drugs

Figures within parentheses= expected values (to be found if the occurrence of the events were independent of the choice of drug)

	Phenprocoumon (53 courses)	Bishydroxy coumarin (33 courses)	Warfarin (28 courses)
Average duration of treatment (weeks)	88 6	135 9	247 2
No of bleeding episodes*	21 (14 9)	21 (14 2)	9 (21 9)

* The distribution between the three drugs is significantly different from that expected (χ^2=13 33 p<0 01)

the three drugs A hypothetical (expected) value was calculated assuming that the choice of drug does not influence the frequency of bleeding The average duration of treatment is longer for warfarin but warfarin resulted in less than half the expect ed number of bleeding episodes

Fig 3 shows the relation between the disease which led to anticoagulant therapy and the occur rence of bleeding and thromboembolic manifesta tions Female patients with rheumatic heart disease had relatively many bleeding episodes and patients with peripheral arterial disease experienced most of the thromboembolic manifestations (Table IV) Twelve of the 114 patients had a diastolic BP above 100 mmHg They had 3 of the 34 bleeding episodes and 6 of the 24 thromboembolic episodes listed Seven patients with diabetes mellitus had another 6 of the 24 and two patients with hypercholesterol

mia experienced a total of 5 episodes of thrombo embolic manifestations Fig 3 gives the relation be tween sex and the occurrence of bleeding and thromboembolic manifestations There is no signi ficant difference (χ^2 test) between the two sexes

Table VI illustrates an attempt to analyse the importance of simultaneous administration of other drugs to patients on anticoagulant treatment. The drugs mentioned are all known to be potential in hibitors or potentiators of coumarin drugs (4 5 6 8 9, 10 11 14) The list includes prescribed as well as self prescribed drugs According to the criteria mentioned above 37 5% of the co-medications with possible interference resulted in such an in terference The column with uncertain effect on the PP% (56 5% of the co-medications) includes situa tions in which the potentially interacting drug was given or withdrawn at a time of unstable anticoagu lant therapy (fluctuating PP% and/or dosage of coumarin drug) and also cases in which the exact time of the start or withdrawal of the concurrent therapy was uncertain In 6% of the possibly in terfering co-medications it was possible to decide that no interference with anticoagulant therapy oc curred Of the patients 46% were aware of possible risk of co medication with sedatives analgesics or anti inflammatory agents

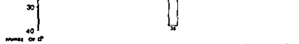


Fig 3 Episodes of bleeding (▨) and thromboembolic manifestations (■) in relation to sex and indication for anticoagulant treatment (□)

DISCUSSION

The present investigation is not a planned therapeu tic trial but should be looked upon as an attempt to contribute to our knowledge of practical problems occurring during anticoagulant therapy in out patients

Patients with hypertension diabetes mellitus or hypercholesterolaemia show a high incidence of arteriosclerotic lesions in the vascular bed (13 18) Of our 114 patients 21 suffered from one of these disorders and they had 17 of the 24 thromboembolic episodes (Table IV)

VI *Drug interactions in 114 anticoagulated* *ents*

	Effect on PP%		
	Observed	Uncertain	No effect
entiation of coumarin effect			
ohol	2		
ic steroids		1	
ad-spectrum antibiotics	16	11	4
ral hydrate	2	2	
rate		1	
ulfiram		1	
antidiabetics	2	3	
phenbutazone	1		
mlbutazone	1	3	
benecid		1	
nidine	2	6	
ylates	7	10	1
roid hormones		3	
bition of coumarin effect			
acids	1	4	
tinocortical steroids	1		
iturates	15	12	1
		1	
trogens	1	3	1
	1	7	
c oil		1	
	1		
	1		
ition and potentiation			
ide	10	27	3
	64	97	10
	37 5	56 5	6

(16) emphasized that thromboembolic festations rarely supervene during coumarin unless the prothrombin time determined he method of Quick is less than twice the l value i e a PP% above approximately 25 We found no thromboembolic manifestations in ts with a PP% below 10 (Table II) Of the des 37 5% occurred while the PP% was e 25 and 25% while PP% was within the utic range (10–25)

nce of haemorrhage Peyman (16) refers to studies covering years with incidences of rhagic complications in out patients from a to more than 40% Pollard et al (17) evaluated ory patients who had been followed for 2 –10½ years and found 61 haemorrhagic ications in 47 (34%) of 139 cases We found orrhagic complications in 34 of 114 cases

lation between bleeding and PP% Peyman (16) found that haemorrhage during anticoagulant therapy was usually associated with an unduly in creased prothrombin time (i e longer than three times the control value using the Quick method of determination) not only on the day of on et of the haemorrhage but also during the previous three days In contrast Pollard et al (17) claimed that the majority of haemorrhagic complications occur while the patients PP%s are within the desired therapeutic range A study by Baugh (1) reveal d n differences in the degree of depression of f or II VII IX and X (all vitamin K-dependent coa ula ir factors) between the patients who bled during anticoagulant therapy and those who d d n t b e d but who had a similar prolongation of the prothrom bin time (determined by Quick s method and Owren s thrombotest) Of the 51 bleeding episodes observed in our study 31 3% occurred while he PP% was below 10 of normal activity The le el f all PP% values determined was significantly l w er in patients with bleeding episodes than in tho without

Physical condition of the patient A Pollard et al (17) hypertension is a re absolute contraindication to anticoagulant Like Coon and Willis (3) and Peyman (16) we no increased tendency to haemorrhagic com tions in patients suffering from mild to mo ra hypertension A high frequency of haemorr ic complications was found in the patien it rheumatic heart disease This is in agreeme t w the fact that patients in this category periodical develop congestive heart failure with hepatic con gestion which tends to increase the risk of bleeding during anticoagulant therapy (3 14 16) Age is significantly correlated with the development of bleedings (3 16 17) In our study about two thirds of the bleeding episodes occurred in the age group 60–69 years Only one third of the patients were between these ages when the therapy was initiated

Drugs used Fremont et al (7) found that warfa rin sodium induced 46 2% and bishydroxycoumarin 37 1% of depressions of the prothrombin complex activity within the therapeutic range ($p=0\ 001$) No difference in the incidence of bleeding episodes was found In our study warfarin resulted in less than half the expected number of bleeding episodes and in a greater incidence (68 3%) of PP% values within the desired therapeutic range than both phenprocoumon (64 8%) and bishydroxycoumarin (61 6%) Our study shows that it was possible to

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Plasma Levels and Effect on Heart Rate and Blood Pressure of Metoprolol after Acute Oral Administration in 12 Geriatric Patients

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"T Metoprolol, a cardioselective β block agent, has been given orally to 12 geriatric pa with moderate hypertension Drug levels of toprolol as well as the effect of the drug on resting rate and BP were studied Metoprolol was ven in a dose of 20 mg and 8 of the 12 patients received a 50 mg dose After the 20 mg dose the drug plasma concentration varied between 5 80 ng/ml (mean 33), and after the 50 mg dose 14 and 212 ng/ml (mean 111) This variation much greater than that seen in earlier studies on hy volunteers and on a group of non geriatric ve patients Plasma half life of metoprolol about 3 5 hours after both the 20 mg and 0 mg dose, this does not differ from the plasma lf life in earlier studies in younger age groups The ility observed in the study might be explained factorially, e g by different body weight, ab on and/or first pass effect of the drug

any elderly patients react to pharmacological nts in a different way, mostly tolerating them well than younger age groups (10 16 20) This have many causes e g altered gastrointestinal on reduced renal function and changes in cell ane permeability Some of these changes tend to produce higher plasma levels and biological half lives of drugs (23) Further alterations in the reactivity of target organs occur This fact demands thorough clinical s of new drugs in the elderly as well as in and middle aged subjects

The aim of the present study is to give an le of pharmacokinetics and also some phar namic data in a group of geriatric patients drug selected for the study was metoprolol prolol (PINN)=H 93/26 Hässle/Astra Sweden Trade names Seloken® Betaloc® Beloc®) Pharmacologic studies in animals (2) and in man (19) have shown that metoprolol is an adrenergic β_1 receptor antagonist according to the classification of Lands et al (21) Unlike practolol the first in a series of recently developed β_1 receptor blocking agents metoprolol is devoid of intrinsic activity (2) β adrenergic blocking agents have proved to be effective antihypertensive drugs also in elderly patients (11) We considered it of interest to study metoprolol in this respect and also regarding its pharmacokinetics The pharmacokinetics of metoprolol have recently been described in healthy volunteers (18 25) and in hypertensive patients 50–58 years of age (9)

MATERIAL

The study was performed during the spring of 1974 Twelve chronically ill geriatric in patients from a clinic specially designed for long term care six males and six females were chosen according to the following criteria Age more than 60 years Arterial hypertension with β blocking agents as a therapeutic alternative No signs and symptoms of cardiac insufficiency atrioventricular block or myocardial infarction in the last six months severe cardiac enlargement insulin-demanding diabetes chronic obstructive lung disease renal or hepatic diseases The patients are briefly described below The main diagnoses and present medication are given in Table I All subjects were informed about the purpose of the study and had given their consent

Case 1 Male 73 years Parkinsonism since 1970 Vertigo S-creatinine 0 7 mg/100 ml

Case 2 Female 88 years Osteoarthritis of knee joints and arterial hypertension since the 1950s Probable pulmonary emphysema Arterial insufficiency of lower extremities Urinary tract infection S-creatinine 0 6 mg/100 ml

Table I *Main diagnosis and present medication*

Case no	Main diagnosis	Present daily medication
1	Arterial hypertension	L-dopa 3 2 g Benzhexol chloride 6 mg Bendroflumethiazide 5 mg KCl 2 4 g
2	Osteoarthritis of knee joint	Methyldopa 500 mg Indomethacin 75 mg Paracetamol 500 mg
3	Cerebral arteriosclerosis	Hydralazine 75 mg Amitriptyline 30 mg KCl 2 g Thioridazine 10 mg
4	Depressive neurosis	Propoxyphyline 800 mg KCl 9 g Nortriptyline 75 mg Propoxyphene 195 mg
5	Parkinsonism	Bendroflumethiazide 5 mg Digoxin 0 13 mg Emepronium 200 mg KCl 2 g
6	Cerebral arteriosclerosis	Diphenylhydantoin 300 mg Bethanidine 40 mg Chlorthalidone 50 mg KCl 2 g
7	Cerebrovascular accident	Hydralazine 75 mg Aspirin 500 mg Propoxyphene 30 mg
8	Cerebral arteriosclerosis	Indomethacin 40 mg Digoxin 0 13 mg KCl 1 g Nitrofurantoin 200 mg
9	Cerebrovascular accident	Hydralazine 75 mg KCl 9 g Phenylsalicylate 1 5 g
10	Cerebrovascular accident	Chlorthalidone 50 mg KCl 3 g
11	Cerebrovascular accident	Bethanidine 40 mg Diphenylhydantoin 300 mg Calcium carbonate 2 25 mg Diazepam 2 mg
12	Parkinsonism	L-dopa 1 8 g Iron and vitamin agent Alimemazin 20 mg Hydroxocobalamin 1 mg every 2nd month

Case 3 Male 67 years Osteoarthritis of spine hip joints and knee joints Arterial hypertension since 1971 Moderate cerebral arteriosclerosis S creatinine 1 3 mg/100 ml

Case 4 Female 76 years Cerebrovascular accident in 1973 Depressive neurosis in periods since 1973 Severe obstipation S-creatinine 0 8 mg/100 ml

Case 5 Female 85 years Arterial insufficiency of right leg Chronic bronchitis Urinary tract infection in periods Osteoporosis Parkinsonism S-creatinine 0 8 mg/100 ml

Case 6 Male 61 years Arterial hypertension in periods with very high diastolic pressure values Vertigo since 1967 Cerebrovascular accident in Feb 1974 S creatinine 0 8 mg/100 ml

Case 7 Male 74 years Cerebrovascular accident in 1972 and in 1974 Able to walk with support S-creatinine 1 0 mg/100 ml

Case 8 Female 74 years Cerebral arteriosclerosis with vertigo Cerebrovascular accident in 1973 S-creatinine 0 8 mg/100 ml

Case 9 Female 82 years Arterial hypertension since 1967 Cerebrovascular accident in 1970 Wheel-chair bound Maturity onset diabetes S-creatinine 0 7 mg/100 ml

Case 10 Male 72 years Arterial hypertension since 1971 In Jan 1974 cerebrovascular accident Urinary tract infection in 1974 S-creatinine 1 3 mg/100 ml

Case 11 Female 77 years Cholecystectomy in 1960 Cerebrovascular accident in 1960 with no sequelae Osteoporosis with fractures of the spine Urinary tract infection S-creatinine 0 5 mg/100 ml

Case 12 Male 71 years Since 1939 four cerebrovascular accidents Parkinsonism since 1939 Gastric resection in 1952 S-creatinine 1 2 mg/100 ml

METHODS

The subjects had fasted for at least 10 hours when they arrived at the laboratory at 7 30 a m They rested in a supine position for 30 min before the investigation started Before administration of the drug two 10 ml blood samples were drawn from an antecubital vein The subjects were then given a tablet containing 20 or 50 mg metoprolol followed by 100 ml of water Blood was collected in heparin tubes at 15 30 45 60 90 120 180 240 300 360 min 12 and 24 hours after the drug administration and the blood samples were refrigerated. Plasma was separated when the tubes reached refrigerator temperature and was then immediately frozen and stored at −20°C until analysis which was carried out by gas-liquid chromatography based on trifluoroacetylation [17] Before every sampling heart rate and BP in the sitting position were measured

The subjects were resting in the sitting position during the first hour of the study Mild physical activity e g knitting and reading was allowed during the whole investigation

All 12 subjects were given 20 mg metoprolol in a first study Subjects 1 2 3 4 5 6 7 and 10 were given 50 mg metoprolol about one week after the first study

Patient 8 had experienced nausea during the 20 mg dose study when her BP decreased from 185/100 to 150/80 Patient 12 had a decrease in BP from 190/95 to 100/70 and patient 9 from 170/80 to 140/65 after the 20 mg dose The 50 mg dose was not given to patient 11 because of a deterioration of her main disease The blood samples from patient 10 in the 50 mg dose study were accidentally spoiled in the laboratories Hence plasma concentration curves were achieved from 12 subjects after 20 mg and 7 subjects after 50 mg of metoprolol

Statistical analyses were carried out according to Student's *t* test

RESULTS

ıvidual plasma levels during the first 6 hours a single dose of 50 mg metoprolol are shown ig 1

The peak levels varied between 4 9 and 80 2 ng/ he 20 mg single dose study and between 14 0 212 ng/ml after the single 50 mg dose (Table II) peaks were generally reached within 1 5–2 after the administration indicating a rapid ion of the drug It should be noted that the ıric resected patient (no 12) did not differ from of the material in these respects

The elimination half life after the 20 mg dose between 1 5 and 10 hours (3 7±0 6 mean ı E) The corresponding values for the 50 mg se were 1 25–4 5 hours for the five patients for hom an elimination half life could be estimated atient 7 demonstrated a very slow absorption rate 50 mg dose with a t_{max} after 3–4 hours patient 5 displayed about the same concentra of metoprolol at 24 as at 6 hours after drug in ke

II *Patient data and plasma half lives of etoprolol (t½) maximum concentrations ($C_{p\,max}$) time to reach maximum concentration (t_{max}) termined from empirically drawn plasma con tion curves*

Age (y)	B wt (kg)	Metoprolol dose (mg)	t½ (h)	$C_{p\,max}$ (ng/ml)	t_{max} (h)
73	60	20	3 25	4 9	0 75
		50	1 25	105 6	1 0
88	75	20	1 75	6 8	2 0
		50	2 0	14 0	2 0
67	73	20	4 0	49 9	1 5
		50	4 5	112 0	1 5
76	53	20	3 5	80 2	1 5
		50	3 75	208 7	1 0
85	45	20	4 75	44 1	1 0
		50	( )	212 2	0 75
61	59	20	4 0	24 2	1 0
		50	3 5	61 0	2 0
74	67	20	10 0	47 4	1 0
		50	(*)	66 5	3 0
74	52	20	2 5	24 0	0 75
8?	56	20	1 5	6 4	2 0
72	65	20	2 5	12 9	0 75
77	80	20	3 5	56 5	0 75
71	46	20	3 0	42 1	2 0

ʼo clear-cut elimination phase was obtained

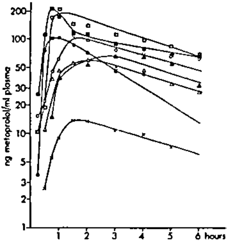


Fig 1 Individual plasma levels during acute administra tion of 50 mg metoprolol tartrate

Effect on resting heart rate

Fig 2 shows the effect of 20 and 50 mg of met prolol on the resting heart rate during acute ministration Maximum effect was obtained hours after administration The average reducti n of the heart rate was 16±4 (range 0–28) and 19±3 beats/min (range 8–36) for the 20 and 50 mg dose respectively when calculated on the eight subj cts given both 20 and 50 mg metoprolol Wh n cal culated on all 12 subjects given 20 mg of meto prolol the average reduction was 17±3 beats/min (range 0–38) The effect on heart rate was signifi cant 3 hours ($p<0\ 01$) but not 4 hours after the administration The effect on heart rate during the first 90 min might be somewhat overestimated as a certain decrease can be expected in any event during this period This was for instance the case

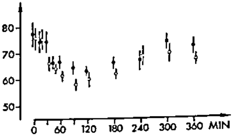


Fig 2 Heart rate after acute administration of 20 mg (●) and 50 mg (○) metoprolol tartrate (mean ±S E.M n=8)

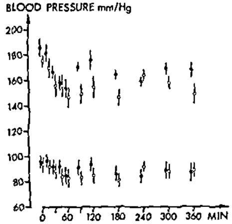


Fig 3 Systolic and diastolic BP after acute administration of 20 mg (●) and 50 mg (O) metoprolol tartrate (mean ±S E M n=8)

in a study on middle aged hypertensive patients by Bengtsson et al (9)

Effects on arterial blood pressure

The average systolic and diastolic BPs during the first six hours after administration of single 20 mg (only the eight subjects who also received 50 mg included) and 50 mg doses of metoprolol are shown in Fig 3 The figure demonstrates that the acute administration resulted in a significant lowering of the systolic BP at 30 and 60 min after 20 and 50 mg respectively and that the effect persisted for six hours The maximal reduction was 25±12 mmHg after 20 mg and 30±10 mmHg after the 50 mg dose The time for maximal effect was 1 hour and 3 hours respectively No significant effect on the diastolic BP was obtained in any of the studies

DISCUSSION

After oral administration drugs are absorbed through the intestinal wall and then pass through the liver via the portal vein before reaching the systemic circulation Some drugs are so extensively metabolized in the intestinal wall and/or the liver on their first passage through these tissues that the area under the blood concentration curve is much smaller after oral than after i v administration This phenomenon is called the first pass effect (14 17 26) A drug with a marked first pass effect has a low oral bioavailability which by definition (3) means the fraction of the dose ultimately reaching the systemic circulation in unchanged form

It has been proposed that the liver first pass effect of some drugs is related to a high affini for the drug oxidizing cytochrome P-450 system the liver (4, 6 15 28) Cytochrome P-450 seen to be important for the distribution of the drug i the liver It may act as a specific binding pool in th liver for unmetabolized drugs which enter the liv cell and reach this site either by free diffusion by some transport mechanism

The degree of bioavailability of β receptor ant gonists varies considerably For example propran lol (27) and alprenolol (1 5 22) are extensivel metabolized during their first passage through th gastrointestinal mucosa and the liver Furthermor a pronounced interindividual variation in the bi availability of alprenolol has been observed by vc Bahr et al (5) Metoprolol has an average bioavai ability of about 45% for oral 50 mg doses (18)

In a recent study where 50 mg of metoprol was given to female hypertensive patients 50- years of age (9) the average plasma levels we in good agreement with data from a study whe 50 mg of metoprolol was given to healthy volu teers (18) The interindividual variations of th plasma levels were however markedly greater the patient study with the individual peak plasm level varying between 35 and 125 ng/ml This vari tion seemed to be mainly an effect of dispariti between the individual elimination rates and th first pass effect of the liver In the present stud the interindividual variations of the plasma leve were still more pronounced the individual pea varying between 14 and 212 ng/ml after the 50 m dose This considerable variation cannot be e plained exclusively as a disparity between the ind vidual elimination rates since in the five subjects whom a plasma half life could be estimated the va ues did not differ from plasma half lives achieved the studies referred to above and the variation wa rather small Hence other explanations have to b searched for

The high maximal plasma concentrations reache in patients 5 (212 ng/ml) and 4 (209 ng/ml) after th 50 mg dose might to some extent be due to the lo body weights 45 kg and 53 kg respectively I fact a significant ($p<0.05$) negative correlation wa found between body weight and the $C_{p\,max}$ value achieved in the 50 mg study On the other han no similar correlation was found in the 20 mg stud which comprised a larger material An addition explanation could be that the bioavailability of th drug might be altered in these patients as an effe

of old age For example if a decrease in the ability of cytochrome P-450 to bind the drug during the first pass through the liver occurs with increasing age an increasing amount of unchanged drug would be expected to reach the systemic circulation Also a change in hepatic blood flow could alter the bio availability of drugs Degenerative changes occur ring with increasing age in the gastrointestinal tract would be expected to impair drug absorption

Although so far no studies seem to have been carried out comparing the rate of drug absorption and total recovery in patients of different ages there are reports that the absorption of some sub stances is reduced and/or delayed in the elderly and these data can be used to infer alterations in drug absorption (7 8 13) The low maximal blood concentrations noted for e g subjects 2 after 50 mg and 2 and 9 after the 20 mg dose could be explained on the basis of changes in the factors which in fluence the absorption (7) e g a decrease in the production of gastric acid with a corresponding de crease in drug solubility a reduced me enteric blood flow or a reduction in size of the absorbing surface

For some of the subjects a good correlation was found between the $C_{p\,max}$ values achieved on the 20 and 50 mg doses respectively (Table II) For others there was disagreement between the two values For example patient 1 had a $C_{p\,max}$ value on the 50 mg dose which was about 20 times higher than on the 20 mg dose No explanation has been found for this pronounced discrepancy but a saturable metabolism during the first pass through the liver might be a contributing factor A similar but less pronounced discrepancy between 20 and 50 mg doses for patient 5 could perhaps be explained by a saturable metabolism whilst a delayed absorption after the 50 mg dose could account for the small difference between the two $C_{p\,max}$ values in pa tient 7

An additional explanation for the large inter individual variation in plasma levels could be inter action of the various drugs taken (Table I) with the pharmacokinetics of metoprolol For example the subjects have been treated with potential in ducers (paracetamol phenytoin propoxyphene) as well as inhibitors (amitriptyline nortriptyline) of cytochrome P-450 mediated drug metabolism Treatment with anticholinergics and/or laxatives (not indicated in Table I) might interfere with drug absorption

The primary purpose of the present study was to investigate the pharmacokinetics of metoprolol in elderly patients Therefore the effect of placebo on heart rate and BP was not included An attempt was however made to correlate plasma levels to clinical response mainly the effect on heart rate The flat plasma level response curve described for β receptor antagonists (24) is probably one reason (of several) why no such correlation was achieved (correlation coefficients $r=0\ 27$ and $r=0\ 41$ in the 20 mg and 50 mg experiments respectively which did not differ significantly ($p>0\ 05$) from zero)

It cannot be claimed of course that the patients in this study are representative of all geriatric pa tients or that the results achieved are exclusively dependent on old age For example the possible interaction of concomitant drugs must be con sidered However the patients represent common elderly patients with chronic diseases with a vary ing often multifactorial clinical picture This re port provides an example of a different clinical pharmacological behaviour of elderly patients with chronic disease and underlines the need for clinical trials of drugs also in such patients

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Clinical Diagnosis in Patients with Smooth Muscle Antibodies

A Study of a One year Material

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ABSTRACT Out of 17 109 sera tested for autoantibodies by indirect immunofluorescence, 236 contained smooth muscle antibodies (SMA) with a titre of ≥1/25. The majority of these sera, from 190 patients, reacted both with smooth muscle and renal glomeruli and the specificity of these SMA is against actin. 91% of high titred sera (≥1/100) with IgG antibodies giving this staining pattern were derived from patients with chronic inflammatory liver disease, mainly chronic active hepatitis. In the group with a titre of 1/25, non liver diseases such as joint diseases were more common and liver conditions occurred only in 55%. Sera with SMA of IgM class were mostly derived from patients with acute viral hepatitis.

Smooth muscle antibodies (SMA) were first reported in sera from patients with chronic active hepatitis (CAH) (15). Later on they have been proved to occur transiently in acute viral hepatitis (8) and in certain other viral diseases not necessarily involving the liver (13, 20) and in Mycoplasma pneumoniae infections (2). SMA may also occur in rheumatoid arthritis (5) and malignant disease (28, 29).

Sera containing SMA at a high titre (>1/80) are reported to be derived usually from patients with CAH (4, 12). In this disease the SMA are mainly of IgG class, whereas they are predominantly of IgM class in acute infections. SMA occurring in CAH are directed against actin (10, 16) but in non liver disease the specificity of SMA has not been fully elucidated.

The aim of this investigation was to study the diagnostic significance of SMA and the possible relationship between SMA titres and different diseases. Information was collected about the diseases of the patients whose sera contained SMA at a titre of ≥1/25 in the routine testing for autoantibodies.

MATERIAL AND METHODS

During 1973, 17 109 sera were sent to the Department of Immunology at the National Bacteriological Laboratory for routine testing of autoantibodies. Specimens were received from most parts of Sweden except from the cities of Linköping, Stockholm and Västerås with own laboratories. Sera from 100 healthy blood donors were tested as controls.

Autoantibodies were demonstrated by indirect immunofluorescence (IFL). Cryostat sections of human thyroid (acetone fixed), rat stomach (unfixed) and rat kidney (unfixed) were used as antigens. Sera were heated at 56 C for 30 min and then tested at a dilution of 1/10. If positive they were also examined at dilutions of 1/25, 1/100 and 1/400. The following fluorescein isothiocyanate (FITC) conjugates were employed: 1) a polyspecific sheep anti human Ig (SBL), 2) sheep anti IgM (Wellcome Laboratories, Beckenham, England), 3) sheep anti IgG (Wellcome). The optic system used was a Zeiss fluorescence microscope equipped with an HBO mercury lamp and a dry darkfield condensor using primary filter BG 3 and secondary filter 44.

Altogether 236 sera from 190 patients (136 females, 54 males) contained SMA with a titre of ≥1/25 (1.4%). Excerpts from the patients' medical records were sent for. The information received was sufficient to be evaluated in 186 cases and all but one of them had been examined in hospital. The clinical and laboratory data on the patients' diseases were compared with the diagnoses established by the clinicians. Particular attention was paid to the presence or absence of liver disease.

Twenty sera from patients with non liver disease but with the same reactivity in IFL as the SMA ser

Table I. *Diagnosis of patients with antibodies of IgG class reacting with smooth muscle and renal glomeruli*

NUD=non ultra descriptus

	Titre (end point) of reaction with smooth muscle			Total no. of cases
	1/25	1/100	1/400	
Liver diseases				
Chronic active hepatitis				
Verified by liver biopsy	15	19	4	38
Probable	10	8	3	21
Chronic persistent hepatitis	3			3
Drug associated hepatitis	2			2
Cholangiohepatitis	3			3
Hepatitis NUD	18	7		25
Primary biliary cirrhosis		1		1
Carcinoma of the gall bladder with liver metastasis	1			1
Alcoholic cirrhosis	2			2
Non-liver diseases				
Rheumatoid arthritis	11			11
Chronic polyarthritis	10			10
Systemic lupus erythematosus	3	1		4
Scleroderma	2			2
Collagenosis NUD	3	1		4
Malignant disease	2			2
Mononucleosis infection	1			1
Miscellaneous	13	2		15
				145*

* 103 females, 42 males.

patients with CAH were randomly selected and absorbed with actin. The procedure has been reported previously (16).

RESULTS

Sera from 172 patients reacted both with smooth muscle and with renal glomeruli. The antibodies were predominantly of IgG class in 145 and of IgM class in 27 sera. Diagnoses and titres of SMA are shown in Tables I and II. Sera from 14 patients reacted with smooth muscle but not with renal glomeruli. Their clinical diagnoses are listed in Table III.

The disease was classified as CAH verified by liver biopsy in 38 patient (27 females, 11 males) with SMA of IgG class (Table I). 15 of them also had signs of cirrhosis.

Twenty-one additional patients (16 females, 5 males) probably suffered from CAH but th diagnoses were not supported by biopsy findings. They all had signs of liver disease existing for more than 6 months with marked elevation of trans aminases and serum γ globulin. The disease ha been diagnosed clinically as CAH in 16 of the 2 patients. The other 5 were diagnosed as non alcoholic cirrhosis. Penetration of the hospital re cords, however, revealed that the disease could b regarded as long standing CAH which had been i progress for more than 10 years in 4 of the cases. Thirty of the 59 patients with proved or probabl CAH also had antinuclear antibodies (ANA).

Chronic persistent hepatitis, verified by biopsy was present in 3 cases. Drug associated hepatitis i 2, one due to sulphonamide and the other probably to hydralazine. Cholangiohepatitis was reported i 3 cases, all of them with concomitant chronic in flammatory bowel disease.

The nature of the liver disease was not suffi ciently defined in 25 of the cases and their disease were listed as unspecified hepatitis—hepatitis NUI (non ultra descriptus). In 13 of these patients th clinical diagnosis of CAH or probable CAH ha been made but this was not verified by liver biops

Table II. *Diagnosis of patients with antibodies o IgM class reacting with smooth muscle and rena glomeruli*

NUD=non ultra descriptus. HB$_s$Ag=hepatitis B surfac antigen. CMV=cytomegalovirus

	Titre (end-point) of reaction with smooth muscle			*Total no. o cases*
	1/25	1/100	1/400	
Liver diseases				
Acute viral hepatitis				
HB$_s$Ag pos	7			7
HB$_s$Ag neg.	13			13
CMV pos	1			1
Chronic active hepatitis	1			1
Hepatitis NUD	1			1
Non-liver diseases				
Mycoplasma pneumoniae infection	1			1
Pyrexia of unknown origin	1			1
Myalgia	1			1
Systemic lupus erythematosus		1		1
				27

Table III *Diagnosis of patients with antibodies of IgG class reacting with smooth muscle but not with renal glomeruli*

NUD non ultra descriptus

	Titre (end point) of reaction with smooth muscle			Total no of cases
	1/25	1/100	1/400	
Rheumatoid arthritis	4			4
Systemic lupus erythematosus	1			1
Scleroderma			1	1
Dermatomyositis	1			1
Polymyalgia rheum	1			1
Myalgia	1			1
Collagenosis NUD	1			1
Myocarditis acuta	1			1
Cystopyelitis recidiv	1			1
Lymphosarcoma	1			1
...culosarcoma	1			1
				14

the clinical and laboratory findings were not typical

Rheumatoid arthritis and chronic polyarthritis common diagnoses among the patients with liver disease (Table I) Two patients had a malignant disease one a lymphatic leucaemia and other a liposarcoma

Absorption with actin of 20 SMA positive sera patients who had non liver diseases abolished reaction with smooth muscle and renal

It can be concluded that 91% of the cases with of IgG class at a titre of ≥1/100 had a liver disease mainly CAH (Table I) This was in contrast the distribution of diagnoses of those with a titre of 1/25 of whom only 55% had liver diseases Fifteen (60%) of the 25 patients with CAH and SMA in titre of 1/25 had received steroid treatment whereas only 10 (29%) of 34 with CAH and a titre of 1/100 were treated

The etiology of the CAH was unknown in most patients Hepatitis B surface antigen (HB Ag) was reported in only 2 cases both with SMA titre of 1/25 The laxative oxyphenisatin could be the cause in 3 cases with CAH (one with SMA titre of 1/400 and 2 with 1/25) and in 2 cases with hepatitis NUD both with SMA titre of 1/25)

The majority of the patients with SMA of IgM class suffered from HB_sAg positive or HB_sAg negative acute viral hepatitis (Table II) One case with cytomegalovirus and another with Mycoplasma pneumoniae infection were also found All patients with acute infections and SMA of IgM class had titres of 1/25

None of the 11 patients whose sera reacted only with smooth muscle (Table III) had a liver disease

Two out of 100 normal blood donor sera reacted with smooth muscle and renal glomeruli at a titre of 1/10 and none at a titre of 1/25

DISCUSSION

SMA of low titres have been reported in 11–18% of sera from healthy blood donors (2 25) and this incidence is not influenced by sex or increasing age (14, 25) Out of 100 blood donors sera tested here only 2% were found to be SMA positive at a titre of 1/10 when tested in the same way as the patients sera This comparatively low incidence was probably due to the microscope equipment we use for routine testing i e substage illumination and dry darkfield condensor This equipment of moderate sensitivity is chosen to detect mainly antibodies of presumed diagnostic significance Moreover the transmittent light used gives a sharper contrast and reveals staining of glomeruli better than incident light Our experience however has been that sera which contain SMA only at a titre of 1/10 are derived from patients with a great variety of diseases Therefore a SMA titre of 1/10 is considered of uncertain diagnostic significance and only those with a titre of ≥1/25 were included in this investigation Tests for autoantibodies are performed particularly in patients with suspected connective tissue diseases and rheumatoid thyroid or chronic inflammatory liver conditions Thus the present clinical material was selected The effect of the selection of sera was also that there were few samples from patients with acute viral infections and malignant diseases conditions in which SMA can appear

SMA positive sera from patients with CAH also react with structures in non muscular cells e g renal glomeruli (30) thyroid epithelial cells (1) liver cells (7) and lymphocytes (6 10) These broad reacting SMA are directed against actin (10 16) which is one of the contractile proteins present in both muscular and non muscular cells (22) Twenty randomly selected sera with IgG antibodies reacting with smooth muscle and renal glomeruli from pa

tients with non-liver diseases also showed anti actin specificity

SMA of IgM class that occur in infectious mononucleosis (unpublished data) and in infections due to Mycoplasma pneumoniae (2) are also directed against actin

A minority of sera reacted only with smooth muscle Preliminary investigations of such sera showed that they can be directed against other contractile proteins than actin (21) The disease in these patients was listed separately and none of them had a liver disease

CAH is a clinical entity first described in 1950 (27) characterized by a chronic progressive liver disease with superimposed episodes of activity (31) The characteristic histological appearance is that of chronic aggressive hepatitis (3) The patients with CAH found in this investigation were divided into two groups In one group the diagnosis was supported by liver biopsy In the other group CAH was considered probable because of signs of progressive non-alcoholic liver disease continuing for more than six months markedly elevated transaminases and serum γ-globulin values The diagnosis however had not been verified histologically Several of the patients with CAH also had signs of cirrhosis which can be regarded as a consequence of the disease (24)

High-titred (≥1/100) sera with SMA of IgG class were usually derived from patients with chronic inflammatory liver diseases Most of these patients had definite or probable CAH Occasionally however high titres were also found among patients in whom the diagnosis of CAH could not be established at the reevaluation of the data although this had been the clinical diagnosis in several cases

Treatment of CAH with steroids can result in decrease in SMA titres (17) Such a tendency was also seen in this material as treated patients were more common among those with a titre of 1/25 than with a titre of ≥1/100

Doniach (4) has described six subgroups of CAH based on the differences in possible initial triggering factor sex age type and titre of autoantibodies The majority of patients with CAH in this investigation fits in with her fourth subgroup where the causal agent is unknown. In this group there is a female preponderance, presence of high titres of ANA SMA and considerable elevation of serum IgG The increased frequency of HL-A 1 and 8 (9 11 18 19) in such patients indicates that there might be a genetic predisposition for an extraordinary immune response resulting in a disease

Another group of CAH can be associated with hepatitis B infection SMA are then usually either not present or of low titre (18 26 32) The fact that only 2 patients with HB_sAg-positive CAH both with SMA at a titre of 1/25 were found might be related to this

CAH has been reported after use of the laxative oxyphenisatin (23) A few cases of this kind were also found in the present material

The diagnoses among the patients with non-liver diseases might mirror the primary selection of the clinical material as patients with joint diseases were common However it can be noted that titres of >1/25 were rare among such patients

Some cases of acute viral hepatitis and one with Mycoplasma pneumoniae infection and SMA of IgM class were found This exemplifies the known occurrence of SMA in certain acute infections

From the present investigation it can be concluded that SMA of IgG class at a titre of ≥1/100 were mostly found in patients with CAH SMA at a titre of 1/25 could appear in several conditions The finding of SMA however should initiate examination for liver disease

ACKNOWLEDGEMENT

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Serum Tnglycerides and Fatty Acid Incorporation into Human Adipose Tissue (FIAT)

Their Relations with Adipose Tissue Characteristics and Glucose Tolerance

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ABSTRACT Fatty acid incorporation into adipose (FIAT), the metabolic process assimilating plasma triglyceride fatty acids liberated by lipoprotein lipase was recently found to be lower in hyper than in normotriglyceridaemia In the present report the relation of FIAT to glucose tolerance adipose tissue morphology and fatty acid composition has been studied in a population of men with normo- and hypertriglyceridaemia, using needle biopsy specimens In addition the associations between plasma triglyceride concentration and these factors as well as FIAT were examined by statistical FIAT and GLIAT (glucose incorporation into adipose tissue) activities per cell were positively correlated with fat cell diameter but not with cell number FIAT activities per cell and per cell surface area were lower in hyper than in normoglyceridaemic subjects The k value of the i v glucose tolerance test and glycerol release from adipose tissue did not correlate with FIAT or GLIAT activities The proportion of stearic acid in adipose was negatively correlated with the serum triglyceride level and with fat cell diameter but positively correlated with FIAT Linolenic acid in adipose tissue correlated positively with the k value The negative correlation between serum triglycerides and FIAT remained when the other variables which were significantly correlated with FIAT or the serum triglycerides were entered in partial correlation analysis These results suggest that although low FIAT activity is related in part to other characteristics it occurs in hypertriglyceridaemia independent of glucose tolerance or various characteristics in fat With serum triglyceride concentration as dependent variable stepwise regression analysis was performed entering all other variables as independent ones The highest multiple R value was 0 76 ($p<0$ 001) and it was obtained with three adipose tissue parameters FIAT (or GLIAT) content of linolenic acid and of stearic acid The other parameters did not give rise to any further improvement in the prediction of the serum triglyceride concentration which is better than 50% ($R^2=0$ 57)

The level of plasma triglycerides is determined by the supply of exogenous fat by the production of triglycerides from the liver and by the removal of triglycerides from blood to peripheral tissues such as adipose and muscle tissue A low fractional removal rate is often found in patients with various types of hypertriglyceridaemia (14 22 41) In the removal process the important role of the lipoprotein lipase system(s) (LLA) in hydrolyzing triglycerides to fatty acids has been emphasized (22 39 42) Very low activity of LLA is characteristic of the rare type I hypertriglyceridaemia (isolated chylomicronaemia) (22 26 33) The activity of postheparin (protamine/salt sensitive) LLA (13 22 33) and LLA determined in adipose tissue biopsies (25 37) in other types of moderate hypertriglyceridaemia is however not infrequently within the normal range

In hypertriglyceridaemia due to impaired removal of triglycerides defects other than low activity of LLA might therefore impair removal of triglyceride fatty acids from blood to tissues After hydrolysis of triglycerides by LLA fatty acids have to be taken up by the tissue We recently reported a low rate of fatty acid incorporation into adipose tissue called FIAT in subjects with different types of hypertriglyceridaemia (16 19 20 48 49 50) It was suggested that low FIAT might

Table I *Serum triglycerides body fat and fat cell diameter fat cell weight fat cell surface area and total fat cell number in subjects with various types of hyperlipidaemia (mean ±S E M)*

	Normals	Type of hyperlipidaemia IIA	IIB	III	IV	V
No of subj	42	8	6	9	35	8
Triglycerides (mmol/l)	1 48±0 06	1 87±0 18*	3 50±0 42***	5 52±1 06***	4 26±0 28***	15 45±2 7
Body fat (kg)	12 4 ±0 7	10 9 ±1 1	13 9 ±1 9	15 2 ±1 3	19 3 ±1 1***	13 3 ±1 7
Fat cell diameter (μm)	77 9 ±2 0	80 3 ±4 1	87 0 ±4 0	83 1 ±4 6	90 3 ±1 2***	86 3 ±1 9
Fat cell weight (μg)	0 28±0 02	0 28±0 04	0 36±0 05	0 33±0 05	0 40±0 02***	0 34±0 0
Fat cell surface area ($\mu m^2 \times 10^3$)	20 26±1 00	21 25±2 04	24 88±2 21	22 99±2 26	26 63±0 73***	24 25±1 06
Fat cell number ($\times 10^{10}$)	5 0 ±0 4	4 4 ±0 9	4 1 ±0 7	5 5 ±0 8	4 9 ±0 5	4 0 ±0 5

Degree of significance versus the normal group * $p<0$ 05 ** $p<0$ 01 *** $p<0$ 001

contribute to the development of hypertriglyceridaemia by slowing down the removal of triglyceride fatty acids from blood to adipose tissue (16 19 20 50 51) The glucose incorporation into adipose tissue named GLIAT was also found to be low in hypertriglyceridaemia and closely correlated with FIAT activity (19)

Several metabolic abnormalities in adipose tissue might be related to and perhaps explain the low FIAT and GLIAT activities found in hypertriglyceridaemia In the present study we measure some of these variables and analyze how they influence the negative relationship between FIAT and serum triglycerides

Table II *FIAT GLIAT and glycerol release expressed per gram of adipose tissue per cell and per unit cell surface area in different types of hyperlipidaemia (mean ±S E M)*

	Normals	Type of hyperlipidaemia IIA	IIB	III	IV	V
subj	42	8	6	9	35	8
T ($\frac{nmol}{3}$)/g/h*	122±12	102±17	65±5***	62±6***	80±9***	52±9***
($\frac{nmol}{3}$)/cell×10^6/h	32 0±3 2	29 1±7 0	23 8±3 8	19 3±2 6**	30 6±3 3	18 0±3 1 *
($\frac{nmol}{3}$)/mm^2×10^{-2}/h	1 59±0 16	1 31±0 24	0 93±0 09***	0 83±0 07***	1 16±0 13**	0 73±0 12**
GLIAT						
nmol×2/g/h*	171±15	161±24	95±17**	98±15***	126±14 *	80±15***
nmol×2/cell ×10^6/h	43 8±4 0	43 3±7 9	35 7±9 0	29 3±4 3*	49 0±5 8	27.5±5 5*
nmol×2/mm^2 ×10^{-2}/h	2 17±0 17	2 03±0 30	1 36±0 27*	1 28±0 13* *	1 83±0 21	1 12±0 21**
Glycerol						
nmol/g/h	616±42	447±46*	399±84*	792±142	724±50	616±55
nmol/cell ×10^6/h	174±18	123±24	141±33	210±66	281±19**	212±25
nmol/mm^2 ×10^{-2}/h	8 18±0 74	5 61±0 60*	4 72±1 45**	11 09±2 13	10 63±0 71**	8 63±0 79

* The values were presented in a previous paper (19) and obtained on the same population as investigated in this study Statistical calculations were determined on logarithmically transferred values and by formulas considering differences in number and in variance * $p<0$ 05 ** $p<0$ 01 *** $p<0$ 001

Table III *Correlations between body fat (BF) fat cell diameter (FCD) fat cell weight (FCW) fat cell number (FCN) and FIAT GLIAT and glycerol release (Glyc) per gram of fat in subjects with different types of hyperlipidaemia*

	FCD	FCW	FCN	FIAT	GLIAT	Glyc
BF	52***	48***	29**	− 30**	− 24*	− 01
FCD		97***	− 54***	− 31* *	− 30**	05
FCW			− 52***	− 24	− 23	05
FCN				− 01	09	− 05
FIAT					78***	01
GLIAT						09

$p<0\,05$ ** $p<0\,01$ *** $p<0\,001$

In a previous study (19) we found that the calculated amount of body fat was negatively correlated with FIAT and GLIAT The amount of body fat is determined by the fat cell size and the fat cell number (5 7 9 11 38 45) In this study the relationship between FIAT and GLIAT activities and the amount of fat is further analyzed by determining fat cell size and number to find out which of these variables correlates most strongly with FIAT and GLIAT activities

In our previous studies (19) we also found a strong positive correlation between GLIAT and FIAT We discussed the possibility that impaired glucose metabolism might explain low FIAT activity since glucose intolerance and/or hyperinsulinaemia are common findings in hypertriglyceridaemia (1 3 17) and in obesity (3 9 11) due to enlarged fat cells (5 7 9 11) In the present study an i v glucose tolerance test (IVGTT) is performed to investigate whether any relationship could be found between glucose tolerance and FIAT–GLIAT activities

We recently found that the percentage content of linolenic acid in subcutaneous adipose tissue was negatively correlated with serum triglyceride levels and positively correlated with the k value of the IVGTT (18) It was suggested that the metabolism of fatty acids in adipose tissue might play a role in the pathogenesis of glucose intolerance and hypertriglyceridaemia In the present study the fatty acid spectrum of adipose tissue glycerides is determined to find out whether linolenic acid or other fatty acids are related to FIAT–GLIAT activities or to other adipose tissue characteristics

The data were analyzed by multiple as well as stepwise regression analysis to determine which factors were most closely associated with the FIAT and GLIAT values and which were the most important in predicting the serum triglyceride level

SUBJECTS

One hundred and six middle aged apparently healthy male subjects and three patients with non-acute diseases and type V hyperlipidaemia participated in the study

Table IV *Regression lines for equations for the linear regression of FIAT GLIAT and glycerol release per cell (y values) on the fat cell diameter (x values) in normoglyceridaemic (normals+type II A) and hypertriglyceridaemic (types II B+III+IV+V) subjects*

	Normals (N)+II A (n=50)		II B+III+IV+V (n=58)		Difference (N+II A) vs (II B+III+IV+V)
	Regression line	r value	Regression line	r value	
FIAT/cell	$y=0\,83x-33$	0 51***	$y-0\,71x-37$	0 36 *	$p<0\,01$ $F=6\,63$ d f 2/104
GLIAT/cell	$y=1\,12x-44$	0 56 **	$y=1\,19x-64$	0 36 *	$p<0\,01$ $F=19\,5$ d f 2/102
Glycerol/cell	$y=5\,24x-244$	0 61**	$y-4\,96x-199$	0 33*	ns $F-0\,78$ d f 2/104

** $p<0\,01$ *** $p<0\,001$ ns=not significant

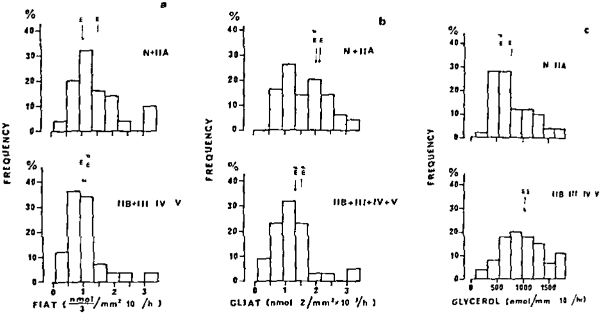


Fig 1 Frequency distribution of FIAT (*a*) GLIAT (*b*) and glycerol per fat cell surface area (*c*) for normo- (*N+IIA* $n=50$) and hypertriglyceridaemic (*IIB+III+IV+V* $n=58$) subjects Median and mean values

Details of these subjects have been published concerning recruitment age fasting serum triglyceride and chol esterol and different lipoprotein levels calculated total body fat and adipose tissue characteristics such as FIAT GLIAT and glycerol release in vitro (19) The variables above were determined in all the 109 subjects as were fat cell size and number In many of the subjects selected at random additional measurements were made IVGTT was performed on 77 subjects of whom 27 had normal lipids

In 62 men 32 of them with different types of hypertriglyceridaemia the fatty acid spectrum of adipose tissue glycerides was determined In 43 of the 62 males also an IVGTT was performed 29 of them were hypertriglyceridaemic

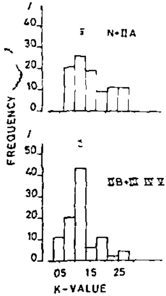


Fig 2 Frequency distribution for the *k* value of the intra venous glucose tolerance test Normo- ($n=33$) and hyper triglyceridaemic ($n=44$) subjects were grouped together Median and mean values

METHODS

The fasting concentration of serum triglycerides (32) and cholesterol (12) and type of hyperlipidaemia (2 15) were determined in the morning after an overnight fast on the same day as the biopsy was taken for the FIAT deter mination Body fat was calculated from anthropometric data (37) Fat cell diameter was estimated according to Sjöstrom et al (44) The diameter for one subject is determined by measuring the diameter for 100 cells and then calculating the mean value and the standard devia tion (S D) This S D for each subject amounts to about 15–20% of the mean value S D is also used in a formula according to Zinder and Shapiro (54) to calculate mean fat cell surface area and mean fat cell weight for each subject Total fat cell number was obtained by dividing the calculated amount of body fat by mean fat cell weight FIAT GLIAT and glycerol activities per cell and per unit cell surface area were calculated FIAT GLIAT and glycerol release were determined with a micro-method in which needle biopsy specimens of subcutaneous fat obtained from the lower abdominal wall are incubated in vitro FIAT is determined as the incorporation of ^{3}H labelled palmitic acid into the fatty acid part and GLIAT as the incorporation of ^{14}C labelled glucose into the glycerol part of extracted di and triglycerides (52)

Table V *Serum triglycerides body fat fat cell diameter and k value of the intravenous glucose tolerance test in different types of hyperlipidaemia (mean ±S E M)*

		Type of hyperlipidaemia				
	Normals	II A	II B	III	IV	V
No of subj	27	6	5	6	31	2
Triglycerides (mmol/l)	1 46±0 08	1 93±0 22	3 46+0 51***	5 63±1 62 *	4 33±0 31*	13 43±3 52
Body fat (kg)	12 0±0 9	10 7±1 0	14 1±2.3	10 7±2 2	19 6±1 2	16 5±0 1
Fat cell diameter (µm)	79±3	82±5	86±5	82±7	89±1**	85+1
IVGTT (k value)	1 67±0 15	1 47±0 22	1 07±0 26	1 21+0 15	1 23±0 01	1 10+0 01

** $p<0\ 01$ *** $p<0\ 001$

The error of determination of fat cell diameter for 30 samples was calculated by the formula

$$S\,D = \sqrt{\frac{\Sigma d^2}{2n}}$$

(*d* difference between two measurements of the same specimens *n*=number of samples analyzed) This error (S D in % of the mean value for all fat cell diameters) was 2 0% and the error for the estimation of mean fat cell weight was 7 1% The errors for the determination of FIAT and GLIAT were about 6% and for glycerol release about 9% as described in detail elsewhere (52) The error for the determination of FIAT and GLIAT per cell (combined error) is then about 10% It should however be pointed out that FIAT–GLIAT activities are determined on different specimens from those used for determination of fat cell diameter and weight although they are taken in the same needle biopsy The actual variation in FIAT–GLIAT per cell is probably much greater since there is a variability in fat cell size of 15–20% *depending on where the specimen is taken* (1) and whether it is representative for the fat used in the incubation

An IVGTT (31) was performed either on the day of the biopsy or within 2 months prior to it The subjects whom the IVGTT had been performed before the biopsy had the same type of hyperlipidaemia on the day of the biopsy as they had when the IVGTT was performed Their weight was stable and they had not received any special diet or drugs before the biopsy The biopsy was taken shortly before the glucose injection Studies were

Table VI *Serum triglycerides body fat fat cell diameter and fatty acid spectrum in adipose tissue in male subjects with different types of hyperlipidaemia (mean ±S E M)*

		Type of hyperlipidaemia				
	Normals	II A	II B	III	IV	V
n	25	4	3	5	20	5
Triglycerides (mmol/l)	1 44±0 09	1 75±0 03	3 56±0 66**	5 48±1 79***	4 32±0 43***	16 79±3 94* *
Body fat (kg)	12 0±1 0	8 7±0 8	11 6+1 9	14 7+1 6	17 8±1 1*	16 2±0 7
Fat cell diameter (µm)	74±3	81±3	86+6	86±6	90±2 **	88±2
Fatty acid						
Lauric	1 73±0 01	1 59±0 15	1 33±0 18	1 74±0 28	1 32±0 13	1 14±0 16*
Myristic	5 44±0 14	4 93±0 18	4 32+0 06	5 31±0 42	4 79±0 12	5 05±0 28
Palmitic	23 71±0 33	22 70±0 24	24 30+1 14	23 42±0 99	24 44±0 46	26 12±0 87
Palmitoleic	6 57±0 19	7 12±0 52	5 68+0 44	7 35±0 47	7 80±0 33 *	8 21±0 72 *
Stearic	5 60±0 24	5 30±0 23	5 51±0 46	4 78±0 42	4 37±0 18* *	4 01±0 42
Oleic	42 55±0 38	43 64±0 98	43 61+0 82	43 21±1 08	44 44±0 35	44 44±0 40
Linoleic	11 94±0 42	12 44±0 88	13 85+0 25	12 17±1 72	11 19±0 46	9 32±0 96
Linolenic	1 81±0 17	1 75±0 38	1 11±0 06**	1 70±0 25	1 32±0 10	1 42±0 20
Arachidonic	0 66±0 10	0 50±0 26	0 17±0 01*	0 32±0 14	0 36±0 05	0 33±0 13
Total						
Saturated	36 50±0 38	34 53±0 57	35 02±0 66	35 45+1 35	34 86±0 49	36 32±1 43
Monounsaturated	49 12±0 41	50 76±1 00	48 87±0 57	50 55±1 08	52 23±0 61	52 65±1 07
Polyunsaturated	14 38±0 55	14 70±0 64	16 11±0 88	14 19±1 82	12 84+0 48	11 07±0 92

$p<0\ 05$ * $p<0\ 005$ *** $p<0\ 001$

Table VII *Correlations between different fatty acids in adipose tissue (%) and serum triglycerides and adi-pose tissue characteristics Only those which were significant ($p<0.05$) are given*

S=total saturated M=total monounsaturated P=total polyunsaturated fatty acids

	12 0	14 0	16 0	16 1	18 0	18 1	18 2
log triglycerides (mmol/l)	−.29*		35 *	42 *	− 49 **	27	− 25
Body fat (kg)	− 34**		41**	29*	− 38 *	28	− 27*
Fat cell diameter (μm)	− 33**	− 43***		27*	−.50 **	31*	
Fat cell weight (μg)	− 28	− 36*			− 43* *	27	
Fat cell number (×10¹⁰)		28*			36 *		
log FIAT$\left(\frac{nmol}{3}\right)$/g/h	32	33**			33**		
log GLIAT (nmol×2/g/h)					27*		
log glycerol (nmol/g/h)					− 28*		

Statistical symbols as in Table I

performed at 8–10 a.m. after an overnight fast The subjects were given 0 5 g glucose/kg b wt. as a single i v injection within 1 1/2 min. Blood glucose concentrations (28) estimated at 20 30 40 50 and 60 min were used to calculate glucose tolerance expressed as the k value from the formula

$$k=\frac{\text{half life time of glucose disappearance}}{0.693}$$

The line for the elimination curve was calculated by the method of least squares from the experimental points plotted on a lin (time) log (glucose) scale and the half life time of glucose disappearance was calculated from this plot.

The fatty acid spectrum was determined on aliquots of a heptane extract (52) of the incubated adipose tissue The heptane phase containing about 1–5 mg of extracted adipose tissue was evaporated under a gentle stream of nitrogen and the fatty acids were hydrolyzed and methylated in 2 ml of sulfuric acid in methanol under nitrogen for 12–16 hours at 60°C The methyl esters were then into 4 ml of petroleum ether (boiling point C) and the phases separated by addition of 2 ml water The petroleum ether phase was evaporated under nitrogen and dissolved in chloroform An aliquot equal to 10–75 μg of fatty acid methyl esters was injected in a Pye Unicam 204 gas–liquid chromatograph equipped with a 2 7 meter 4% EGSS-X column i d. 4 mm. Injection port temperature was 240°C detector temperature 225°C Carrier gas was nitrogen at a flow rate of 35–40 ml/min. A flame ionization detector was used operated with hydrogen at a flow rate similar to the carrier gas. Air flow rate was 350–400 ml/min. The fatty acid methyl esters were separated isothermally at 180°C or 190°C. The peaks were identified according to retention time factors in relation to known standards (30) The NHI A B and D standards and PUFA 1 and PUFA 2 standards as well as the phase and support for the column were supplied by Supelco Pennsylvania USA The separation characteristics of the column and the quantitation procedure were checked every day and fulfilled the criteria described by Horning et al. (30) The retention time of the peaks and the peak areas were determined on an Autolab 6700 digital integrator The nine most common fatty acids (Tables VI and VII) were identified and their total area was called 100% The percentage content of each fatty acid was then determined All chemical agents were purchased from Kebo Stockholm Sweden. Petroleum ether and chloroform were redistilled before use

Statistical calculations were performed according to Snedecor and Cochran (47) and the IBM Library (24) Measurements whose distribution was found to be skewed to the right were transferred logarithmically to normalize their distribution before statistical analyses were performed (47) When calculating the degree of significance of mean values in populations with many compared with few observations ($n_1 \neq n_2$) or with differences in the variance ($\sigma_1 \neq \sigma_2$) in these populations the test introduced by Welch as used by Snedecor and Cochran was used

Stepwise regression analysis was performed according to program BMD02R, Health Sciences Computing facility UCLA 1970 as handled by the IBM 320/155 By applying the technique of stepwise regression analysis an attempt was made to indicate the contribution of each of the measurements to the overall correlation with the triglyceride level. There are several ways of handling stepwise regression analysis depending on what questions are asked (24 47) In this study it was decided to start with the measurement having the highest correlation with the triglyceride level and then successively add the factor which contributed next most to this correlation when the common variation with the previous factor had been eliminated

RESULTS

Adipose tissue morphology

Fat cell diameter (Table I) was about 80 μm in normals and type II A subjects and mean values of 83–90 μm were obtained in the various hypertriglyceridaemic groups The highest mean value 90 μm was obtained in type IV subjects The mean fat cell weight (Table I) was calculated and the values were about 0 30–0 40 μg in all groups The highest values were found in those with type IV

)	70 4	S	M	P
~	− 31*		40**	− 33**
	− 30		33**	
5	− 78*	− 27*	36**	
	− 25*		31*	
		27*		

hypertriglyceridaemia The calculated fat cell surface area (Table I) was significantly larger in subjects with type IV hypertriglyceridaemia than in normotriglyceridaemic subjects The number of fat cells (Table I) in each individual was about 4 5× 10[10] and not significantly different in any type of hyperlipidaemia

The distributional values for serum triglycerides body fat FIAT GLIAT and glycerol release per gram of fat in the whole population were skewed to the right (19) In the present study FIAT GLIAT glycerol release per cell and per unit surface (Fig 1) in the normo- and hypertriglycerid subjects also showed skewed distributions

The mean FIAT and GLIAT values per cell (Table II) were somewhat lower in almost all hyper yceridaemic groups and significantly lower in type III and type V hypertriglyceridaemia The values for glycerol release per cell were higher in IV than in normotriglyceridaemic subjects

The mean values for FIAT activities expressed unit surface area (Table II) were lower in all ypertriglyceridaemic subjects GLIAT per unit ace area was reduced in type III and V Glyc release per unit surface area was lower in type IA and IIB but higher in type IV hypertriglycerid ia.

Several correlation coefficients were calculated Table III) Both fat cell diameter and the number of fat cells correlated positively with the amount of fat FIAT and GLIAT per gram of fat corre negatively with fat cell diameter and weight The correlations between fat cell diameter and FIAT/cell GLIAT/cell and glycerol/cell were $r=$ ($p<0\ 001$) $r=0\ 39$ ($p<0\ 001$) and $r=0\ 42$ ($p<$ 001) respectively When regression lines were between FIAT GLIAT and glycerol expressed per cell and fat cell diameter the lines for normotriglyceridaemic and type II A subjects had a higher intercept on the y axis (activity/cell) than the lines for other hypertriglyceridaemic subjects with respect to FIAT/cell and GLIAT/cell but not glycerol/cell (Table IV) This indicates that at the same fat cell diameter normotriglyceridaemic subjects had higher FIAT/cell and GLIAT/cell values than hypertriglyceridaemic subjects

The correlations between serum triglycerides and FIAT GLIAT and glycerol per unit surface area were $r=-0\ 39$ ($p<0\ 001$) $r=-0\ 39$ ($p<0\ 001$) and $r=0\ 27$ ($p<0\ 05$) The correlation between the serum triglycerides and FIAT and GLIAT per gram were $r=-0\ 47$ and $r=-0\ 46$ These correlations were insignificantly reduced to $r=-0\ 41$ ($p<0\ 001$) and $r=-0\ 41$ ($p<0\ 001$) when the influence of fat cell diameter was eliminated in partial correlation analysis

The product of the estimated average fat cell weight multiplied by the total calculated number of fat cells equals the calculated total body fat In the stepwise regression analysis cell weight and number as well as FIAT GLIAT and glycerol per gram were included to determine which of these parameters correlated most strongly with the serum triglycerides which was the dependent variable In this analysis either FIAT or GLIAT was chosen first since these variables had the highest correlation with the triglycerides Fat cell weight was the next most important determinant increasing the multiple R value from 0 47 to 0 55 for FIAT and from 0 46 to 0 54 for GLIAT These multiple R values are highly significant Glycerol and fat cell number were picked out last and did not contribute significantly to the correlation as indicated by the F ratio of the individual parameters in the total equations

The intravenous glucose tolerance test

The number of subjects in each type of hyper e lipidaemia and the mean values for serum tri ed glycerides body fat and fat cell diameter (Table cell of the men who had an IVGTT were representati cts for the whole population (Table I) The k values IAT all normotriglyceridaemic (N+II A) subjects and were all hypertriglyceridaemic subjects showed a d cerid bution skewed to the right (Fig 2) The mean lower of the IVGTT were about $k=1\ 5$ in the concern lipidaemic and type II A group and lower reported 1 2) although not significantly lower in the

The negative correlation between serum triglycerides and FIAT activity was almost unchanged when fat cell diameter was kept constant in partial correlation analysis. This and the above findings by us suggest that although fat cell characteristics are related to the FIAT–GLIAT activities, other factors in the fat cell might also contribute to the low FIAT–GLIAT values found in hypertriglyceridaemic subjects.

Several findings argue in favour of calculating adipose tissue activities per gram rather than per cell in our studies. The specimens in which activities are determined probably contain mainly fat cells. Although gross connective tissue has been dissected away, some of it as well as other cell types may remain in the specimen and contribute to the measured activities. It is then not correct to extrapolate these measured activities to a fixed number of *fat* cells, which furthermore has been calculated on separate specimens probably with different morphology from the pieces used for determination of activities (see Methods). Although it is always difficult to extrapolate results from in vitro studies to be valid for the metabolism in vivo, it is more attractive to measure activities in specimens of fat than in isolated cells since adipose tissue in situ is composed of different cell types individually arranged in different subjects. It is probably only valid to express activities per cell when studying the metabolism of the *adipocyte itself* (cells isolated by collagenase). In such studies one can measure the activity per cell more accurately since it is possible to obtain a representative subsample from the cell suspension for separate analysis of fat cell morphology. These observations together suggest that it is probably neither biologically nor methodologically correct to calculate FIAT–GLIAT and glycerol per cell when relating adipose tissue activities to serum triglyceride levels.

It might be assumed that the total body fat FIAT activity rather than a value from a single site would be the relevant determinant of the serum triglyceride levels. In order to obtain such a total body FIAT value, however, one has to determine the FIAT activity in other regions of fat. Both FIAT and GLIAT activities are 40–50% higher in omental than in subcutaneous fat (52). As long as the proportions of these main fat depots are not known, one must be cautious about interpreting a single value as representative for the FIAT activities in all other fat regions. There was, however, a strong positive correlation between subcutaneous and omental fat activities, both in normo- and in hyperlipidaemic subjects (45).

Glucose metabolism

In previous studies we found that FIAT was closely correlated with GLIAT (79). The question was discussed that acyl dihydroxyacetone formation from exogenously incorporated glucose (GLIAT) and/or its components, was a prerequisite for reesterification of exogenous fatty acids into adipose tissue glycerides, i.e. FIAT activity. It is therefore possible that a disordered metabolism of glucose may cause reduced FIAT function in hypertriglyceridaemic subjects. Some support is given to this hypothesis by the fact that glucose intolerance is frequent in hypertriglyceridaemia (? ?). In this study there was a weak negative correlation between the k value and the triglyceride concentration but no correlation between the k value and the GLIAT or FIAT activities. Furthermore, the negative correlation between FIAT and the triglycerides was not weakened when the k value was kept constant in partial correlation analysis. These findings suggest that low FIAT occurs in hypertriglyceridaemia although the k value is normal. The reason for the lack of correlation between the k value and GLIAT–FIAT is probably the fact that tissues other than adipose tissue, i.e. the liver and muscle, determine the k value to a large extent.

In other studies we found that diabetic patients had very low GLIAT and FIAT activities, but also an increased rate of lipolysis (46). Similarly, by using somewhat different methods, Östman (?) found that patients with poorly controlled overt diabetes had a decreased reesterification and an increased lipolysis rate compared with controls. In his studies the glucose uptake was the same in all subjects but the rate of formation of glyceride-glycerol from exogenous glucose was not determined.

Interestingly, the negative correlation between FIAT and serum triglycerides, although weaker, still exists when GLIAT and/or other parameters are kept constant in partial correlation analysis. These results, however, do not exclude the fact that the metabolism of glucose (or metabolites of carbohydrates) in the adipocyte is of importance for the FIAT process. It may be that acyl dihydroxyacetone phosphate is formed from other precursors such as

pyruvate (43) and/or that other intracellular fatty acid acceptors such as diglycerides or phosphatidic acid are of importance in the esterification of incorporated fatty acids as discussed (23)

Fatty acid spectrum of adipose tissue glycerides

The mean values for the percentage composition of the different fatty acids in normolipidaemic subjects were in agreement with earlier findings as reviewed (21 27) The most striking difference between normo- and hyperlipidaemia was the significantly lower proportion of stearic acid in subjects with type IV hypertriglyceridaemia It should be noted that stearic acid in serum triglycerides correlates positively with serum triglyceride levels (Walldius unpublished observations) whereas the present study has shown that stearic acid in adipose tissue correlates negatively with serum triglyceride levels This may indicate that the metabolism of stearic acid is in some way related to the removal mechanism of triglyceride fatty acids from blood to tissues This is also suggested by the positive correlation obtained between FIAT and the percentage stearic composition of adipose tissue

However several other correlations between the fatty acid spectrum of adipose tissue and the serum lipid levels as well as of fat cell diameter and FIAT–GLIAT functions were also obtained From other studies it is known that different fatty acids have different reesterification rates (23 29) Thus it is possible that *the fatty acids in serum triglycerides* may affect the rate of uptake of individual fatty acids into adipose tissue perhaps through stereospecific enzymatic sites at the LLA step or at later stages inside the fat cell and thereby create a fatty acid spectrum in the fat tissue which reflects mostly the long term effects of the removal process (27) It may also be possible that the various fatty acids in the tissue glycerides known to be mobilized at different rates (29) influence the activity of LLA and of FIAT differently depending on the fatty acid spectrum of adipose tissue An inverse relationship has been described between lipolysis and LLA activity (36) as well as between lipolysis and FIAT activity (40 48 53) but detailed information on the effects of different fatty acids is not available It should be emphasized that the FIAT process here was only estimated with palmitic acid

It was also of interest to find that the adipose tissue content of linolenic acid was closely correlated to the k value (18) This suggests that glucose metabolism is in some way associated with the metabolism of the individual fatty acids especially with the polyunsaturated fatty acids

Several factors were correlated with serum triglycerides *and/or the FIAT activity The negative* correlation between these two variables remained significant when one or several of the other variables such as fat cell diameter and number GLIAT activity and glycerol release percentage content of stearic and linolenic acid and the k value were held constant in partial correlation analysis These results suggest that there are additional characteristics in adipose tissue of hypertriglyceridaemic subjects that have to be identified and measured to understand why their FIAT values are low

Prediction of serum triglyceride concentration

The various relations between serum triglycerides and the different variables were further investigated By applying the technique of stepwise regression analysis an attempt was made to predict the triglyceride level by measuring the contribution of the different factors in a multiple correlation with serum triglycerides When these calculations were performed on subjects in whom fat cell weight and number and the k value as well as the incubation variables were determined it was found that FIAT (GLIAT) and cell weight contributed most but glycerol release and the k value only a little to the multiple correlation with serum triglycerides In those subjects in whom *all* variables were measured and analyzed it was found that fat cell weight did not contribute significantly to the multiple correlation since linolenic and stearic acid and FIAT (or GLIAT) were all stronger determinants of serum triglycerides These three variables together explained 57% (R^2=0 57) of the serum triglyceride variation in the different types of hypertriglyceridaemia *It is also very likely* that lipoprotein lipase activity in adipose tissue contributes to a large extent to these correlations thus further increasing the importance of adipose tissue as a determinant of the serum triglyceride level

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Extreme Digitalis Intoxication

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ABSTRACT A case of massive digoxin intoxication is described The concentration of digoxin in plasma 15 5 ng/ml is one of the highest observed in an individual not having heart disease who survived the intoxication During the first two days there was complete heart block but only moderate hyperkalaemia The advantage of temporary pacemaker treatment under these conditions is emphasized

Digitalis overdosage is a frequent complication of digitalis therapy Several studies (4 8 14) have indicated that about 17–20% of all patients treated with digitalis have symptoms of overdosage and the condition is also considered to be associated with an increased mortality

In contrast to this situation massive digitalis intoxication whether accidental or with suicidal intent is rare The mortality in these situations has been high 20–25% (1 5 6 12 15) There are however few reports of intake of large doses of digoxin by individuals without heart disease in which the concentration of digoxin in plasma is stated The present communication reports a case of massive overdosage of digoxin leading to very high plasma concentration levels of digoxin and serious conduction disturbances

CASE REPORT

A 45-year-old man with pernicious anaemia who was otherwise healthy and weighed 98 kg took 17 5 mg digoxin with suicidal intent On admission to hospital three hours later he was fully conscious but suffered from nausea vomiting and dizziness Physical examination revealed normal results BP was 170/90 mmHg and pulse rate 96/min An ECG showed sinus rhythm with only slight ST T segment depression in the left precordial leads

The patient was transferred to the Intensive Care Unit and during the next few hours he had isolated supraventricular ectopic beats and periods of sinus arrest and increased P-Q time Eight hours after tablet intake complete AV block occurred with a ventricular rate of 35/min Repeated doses of 0 5 mg atropine i v were without effect and a transvenous pacemaker electrode was therefore placed in the apex of the right ventricle and on-demand pacemaker treatment at 80 beat min was started Pacing was continued for more than 24 hours and the complete block then disappeared From the fourth day the patient had normal sinus rhythm without conduction disturbances The ECG tracings showed marked ST segment depression and T wave inversion during the first week but no ventricular arrhythmias were seen during the whole observation period of 6 days Electrolyte status was normal apart from moderate hyperkalaemia during the first two days 5 5 and 5 3 mmol/l respectively Serum magnesium was 0 8 mmol/l Serum creatinine was normal (80 μmol/l) throughout the period of treatment as were the urinary volume and BP The digoxin concentration in plasma determined by radioimmunoassay 10 hours after intake of digoxin was 15 5 ng/ml The digoxin concentration was determined at regular interval (Fig 1)

The patient s general condition was good throughout the period of treatment but he was troubled by nausea tinnitus and dizziness during the first three days No visual disturbances or periods of incoherence occurred The patient was able to leave the hospital in full physical health 11 days after admission

COMMENTS

The toxic effects of large doses of digoxin differ considerably between persons with and without heart disease (5) The case described here demonstrates some of the most typical symptoms of severe overdosage of digoxin in a person previously free from heart disease

When a normal heart is exposed to a very high dose of digoxin it develops AV conduction disturbances with bradycardia and varying AV block and an increased risk of lethal bradyarrhythmias (1 2 5 15) A diseased heart reacts primarily with increased generation of ect

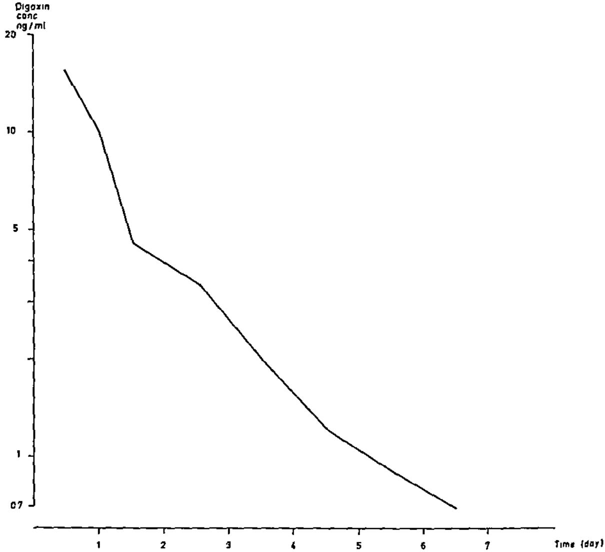


Fig 1 Digoxin concentration in plasma during the first even days after intake of 17 5 mg digoxin

above all from the ventricles leading to various ventricular arrhythmias Atropine has been reported to abolish this digitalis induced AV block in certain cases (10 15) In other cases as in the present patient the extravagal effects of digoxin on the conduction system appear to be the main cause of the AV block Treatment may be diffi cult in these cases especially in view of the great risk of lethal bradyarrhythmias in connection with complete block (1) Phenytoin improves conduction in the AV node and has been successfully used in digitalis intoxication (9) it has also given good results in AV block due to massive doses of digoxin (13)

When AV conduction disturbances occur which cannot be treated successfully with drugs the patient should be provided with a temporary trans venous pacemaker electrode before malignant bradyarrhythmias arise In the case reported here complete block occurred nine hours after intake of digoxin As the block did not respond to atropine the patient was treated with an on-demand pace maker for the next 24 hours and no further arrhythmias were observed No ventricular arrhythmias were seen even during the first 24 hours when the plasma levels of digoxin were very high The initial plasma concentration of digoxin 15 5 ng/ml ten hours after intake of digoxin is one

of the h ghest reported in an ind vidual without heart d sease who *survived the intoxicat on* The normal half l fe in blood for d gox n has been cal culated to be about 31 hours (3) After intake of large doses of d goxin however the half life has been substant ally shorter (7 13 15) Smith and *W llerson (15) found that the half l fe during the* first t o days was about 10 and 21 hours respec ti ely subsequently increasing to normal values In h s case the d goxin concentrat on fell relatively rap dly with a calculated approximate half l fe durin the first two days of about 20 hours During the following two days the half life was about 33 hours By the fourth day the d goxin concentration was less than 2 ng/ml and no AV conduct on disturbances or other arrhythm as were now present

Mass ve doses of d goxin may cause hyperkalae mia probably due to a generalized inhibition of cell membrane ATP ase and consequent altered trans port of certain cations without other electrolyte d sturbances or acidosis occurring (1 2 6 11 12 15) Th s hyperkalaemia may be very pronounced and two cases have been reported in wh ch very h gh doses of d goxin caused refractory hyperkalae m a w th lethal outcome (12 15) In most cases however serum potassium becomes normal when the d *goxin concentration falls* to non *toxic levels* In the case described here only a moderate hyper kalaem a was observed during the f rst two days (in t ally 5 5 mmol/l) despite the very high digoxin concentrat on Thus potassium is contra nd cated u der these condit ons and should *not* be *given* unt l the serum potassium level has been deter m ned and renal funct on tests have been per formed In cases of moderate digoxin overdosage in ind v duals w th heart d sease on the other hand potass um must often be given since hypokalaemia is a frequent f nding under these cond t ons

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Nephrotic Syndrome due to Subacute Glomerulonephritis —Association with Hydrocarbon Exposure?

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BSTRACT A 59 year-old man developed a nephrotic syndrome 40 days after hydrocarbon exposure of 3 days duration Renal biopsy gave evidence f a *subacute proliferative glomerulonephritis* A sting remission was obtained with immunosuppressive therapy The case history is discussed in the ght of recent studies indicating a causal relationship etween hydrocarbon exposure and glomerulonephritis The reversible nature of the disease in the resent case is discussed in relation to the disease i experimental animals induced by a single administration of heterologous antigen

nvironmental factors have won increasing recognition as actual or possible causes of disease he agents that provoke chronic glomerulonephritis re still obscure but in the majority of patients iere are reasons to believe that immunological iechanisms are involved The factors which trigger is abnormal immunological response are however largely unknown

During the last ten years there have been cattered reports of an association between hydrocarbon exposure and rapidly progressive glomerulonephritis (1 4 6) Recently it was shown hat patients with chronic proliferative glomerulonephritis had been exposed to hydrocarbon ignificantly more than patients with other renal iseases (8) All patients hitherto described have iad a *chronically* deteriorating disease course esulting in end stage renal failure In contrast the resent patient developed a nephrotic syndrome lue to biopsy proven subacute proliferative glomerulonephritis 40 days after hydrocarbon exposure The disease healed with immunosuppressive therapy and intermittent proteinuria has been the only sign of residual renal damage at subsequent controls

CASE REPORT

The patient a post-office employee born in 1912 was well until 1970 when he was operated on for perforation of a duodenal ulcer A gastric resection was performed After that he was in excellent health until the end of Oct 1971 at which time he spent three days painting a floor He used 30 l of paint containing diacetone alcohol and ethanol as solvents At the end of Nov he first noted edema of the legs The edema progressed rapidly and was generalized within four days A diuretic was prescribed and some relief was experienced During the first week of Dec the edema could however no longer be controlled There was again a rapid progression resulting in pronounced and generalized edema He gained about 12 kg in weight from the time of the first symptoms until the latter half of Dec

He was referred to the Medical Department in the end of Dec 1971 On admission he was found to have anasarca the edema being most pronounced in the lower half of the body The breath sounds were diminished corresponding to the basal part of the right lung His BP was 120/80 mmHg Otherwise physical examination showed normal results HB was 15 0 g/100 ml WBC normal as a differential count ESR 100 mm/hour Total serum protein concentration was 4 3 g/100 ml albumin 1 7 g/100 ml globulins 2 6 g/100 ml Fibrinogen was 0 8 g/100 ml There was 11% protein in the urine corresponding to a urinary excretion of 10 g/24 g hours The 24-hour urine volume was 1 000–1 500 ml Within 16 hours after the administration of pitressin tannate the urine osmolality reached a maximum value of 980 mOsm/kg Urinary microscopy showed 5 red cells per high power field and a moderate number of granular casts Cholesterol was 540 mg/100 ml triglycerides 306 100 ml Serum creatinine was 1 4 mg/100 ml

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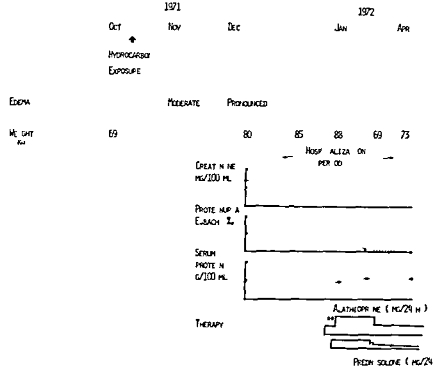


Fig 1 The course of the disease and treatment.

a peak value of 2 ? mg/100 ml on the 10th day in hospital (Fig 1) Normal values were obtained at repeated serum estimations of sodium potassium calcium standard bicarbonate and transaminases A culture from the throat yielded no growth of streptococci The antistreptolysin titer was normal Chest X rays revealed moderate bilateral pleural effusions A kidney biopsy was performed on the 8th day in hospital The microscopic examination revealed changes compatible with the diagnosis of subacute proliferative glomerulonephritis (Fig 2)

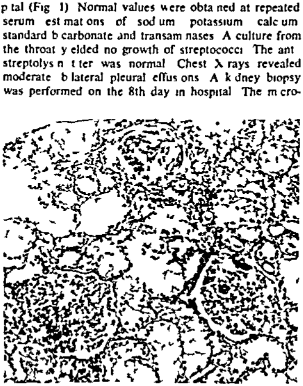


Fig 2 Kidney biopsy Three glomeruli showing hypercellularity capsule proliferation and adhesion between glomerular tuft and capsule Degenerative changes in the proximal convoluted tubules with swollen vacuolated epithelial cells (v Gieson ×107)

Quantitative immune electrophoretic determinations of the plasma and urine proteins revealed increased plasma concentrations of macromolecular proteins such as fibrinogen and α 2 macroglobulin and a pronounced reduction of albumin IgG was 0 3 IgA 0 16 and IgM 0 17 g/100 ml β 1 globulin (C_3) was within normal limits (110%) but β 1_E (C_4) was reduced to 76% of the normal value The urinary protein pattern was compatible with that found in unselective proteinuria No circulating antibodies against glomerular basement membrane (GBM) could be demonstrated with immunofluorescent studies

During the four week stay in hospital the patient was normotensive His weight reached a maximum of 88 kg at the end of the first week The urine output was persistently in excess of one l Treatment with corticosteroids azathioprine and furosemide was started on the 10th day Within one week the proteinuria diminished to 2 8‰ and within two weeks to 0 5‰ His weight also normalized rapidly The patient was discharged at the end of the 4th week on prednisolone and azathioprine

Frequent reinvestigations revealed a continued improvement Trace amounts of protein were found intermittently in the urine Serum proteins and serum creatinine returned to normal The patient was persistently normotensive In Aug 1972 the creatinine clearance was 90 ml/min the same value was obtained in Sept 1973 The prednisolone and azathioprine therapy was gradually discontinued in Oct 1973 In May 1975 BP was 140/80 mmHg ESR 19 mm/hour Hb 14 1 g/100 ml and serum creatinine 1 2 mg/100 ml There was a proteinuria of 0 8‰ Reportedly he was in excellent health

COMMENTS

The evolution of a nephrotic syndrome due to a subacute proliferative glomerulonephritis 40 days after hydrocarbon exposure in the present case may well be a coincidence However the absence of other diseases or disease provoking factors known to be associated with glomerulonephritis makes it necessary to consider such a causal relationship

An association of Goodpasture s syndrome with exposure to petroleum products was suggested by Klavis and Drommer in 1970 (4) They described a 26-year old painter who within two months after a single heavy exposure to a gasoline based paint spray developed pulmonary infiltrates pulmonary hemorrhages and a rapidly progressive glomerulonephritis They were also able to induce a similar disease in rats by chronic exposure to gasoline vapors In 1972 Beirne and Brennan (1) on the basis of interviews reported that six of eight patients with glomerulonephritis associated with GBM antibodies had a previous history of exposure to various hydrocarbon solvents Accordingly a theory was proposed implying chemical injury to the lung or the kidney as the first step followed by autoimmune organ damage

The present case does not fit into the category of patients with rapidly progressive glomerulonephritis associated with GBM antibodies The absence of such antibodies and the benign clinical course contradict the diagnosis of anti GBM glomerulonephritis and would rather suggest an immune complex genesis Before 1975 however nearly all the cases in which hydrocarbons were suspected of being implicated had the peculiarity of being examples of anti GBM glomerulonephritis Also in rats fed with N N diacetyl benzidine a disease could be induced which conformed to rapidly progressive glomerulonephritis in man (3) In 1975 Zimmerman et al (8) were able to demonstrate that patients with proliferative glomerulonephritis had been exposed to hydrocarbon significantly more than patients with a variety of other renal diseases Five of 12 patients with biopsy proven proliferative glomerulonephritis were found to have linear depositions of IgG suggesting GBM antibodies Of the remaining patients four had granular deposits of IgG IgM and C_3 This finding could indicate glomerular deposition of immune complexes Immune complex deposition is supposed to be the most prevalent mechanism leading to glomerulonephritis It is thought to occur with much higher frequency than anti GBM antibodies in acute as well as in chronic glomerulonephritis (5 7)

The benign and limited course of the disease in the present patient contrasts with the chronic and irreversible pattern in the patients described earlier To explain this we suggest as an experimental model a self limiting immune complex disease called by Dixon (2) one shot serum sickness In this disease a single exposure to antigen is followed by production of antibodies antigen-antibody complexes are formed and there is a resulting deposition of immune complexes in the tissues This phenomenon coincides with the appearance of an acute reversible glomerulonephritis

Admittedly the proposed scheme of events leading to the subacute proliferative glomerulonephritis in the present case is highly hypothetical Nevertheless the possibility of hydrocarbon exposure as an important factor in the pathogenesis of glomerulonephritis in whatever form it may appear cannot be ignored The prevalence of hydrocarbons and their recently recognized role as an occupational and environmental hazard warrant increased awareness and further exploration

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LETTERS TO THE EDITOR

Sir

In their paper entitled On the use of renal angiography and intravenous urography in the investigation of renovascular hypertension (Acta Med Scand 198 pp 39–44 1975) Erikson et al reach conclusions which do not agree with our findings

First of all we think it is extremely important to define the terms used The term renovascular hypertension should only be used to identify a condition of renovascular disease etiologically related to a coexisting hypertension Proof of such a relationship is supplied by an abnormal renin production from the kidney involved and preferably also by cure or improvement after nephrectomy or reconstructive surgery It is obvious that an i v urography cannot reveal a renovascular disease per se but only the hemodynamic consequences of a renovascular disease responsible for a renovascular hypertension Erikson et al do not state the number of patients who really suffered from renovascular hypertension according to these criteria It is therefore impossible to draw any conclusions from their data regarding the value of the methods in question According to our experience of 98 patients with proved renovascular *hypertension operated on in 1971* through 1975 i v urography and renal angiography afford considerably more information than stated by Erikson et al

Intravenous urography should be done with the rapid sequence technique The Maxwell modification of i v urography reveals any difference in the appearance time of the contrast medium in the collecting systems of the kidneys which is one of the most important signs of significant renal artery stenosis Even more information is achieved if a large dose of contrast (1 ml/kg b wt) is injected rapidly followed by tomography in the nephrographic phase to reveal impaired circulation locally or in the entire kidney

In a study of 34 patients 23 with unilateral and 11 with bilateral stenosis with proved renovascular hypertension followed postoperatively *for at least one year 27 uro*graphies (79%) showed abnormalities indicating renovascular hypertension Since many of our patients were referred from other parts of the country rapid sequence urogram was not done in all of them

It is true that about 20% of the patients with proved renovascular hypertension have a negative urogram This may be due to bilateral stenosis branch stenosis or multiple arteries The most common cause of a false negative urogram is however unsatisfactory technique and lack of knowledge of the characteristic urographic abnormalities

The angiographic study in hypertension should not only demonstrate the presence of renal artery stenosis but also 1) exactly demonstrate the localization distribution and degree of stenosis 2) demonstrate if possible the hemo*dynamic significance of the stenosis* and 3) demonstrate peripheral vascular or parenchymatous abnormalities which may influence the indication for operation In order to achieve this the angiography should be done with the proper technique In our opinion the Erikson catheter is not well suited for *renal angiography We use a thinner* catheter with a long tapered distal part which makes it less traumatic The catheter is bent in such a way that the tip can be introduced into the orifice of one of the renal arteries thus allowing selective injection in one of the renal arteries and simultaneous injection at an optimal level in the aorta (semiselective technique) Then selective study of both kidneys is performed to delineate the peripheral vessels Magnifying technique is used

Table I demonstrates the most important angiographic parameters and the results of surgery in 28 patients operated on for unilateral renal artery stenosis Renal vein renin was determined in every patient Postoperatively the patients have been followed for at least one year From the data it is evident that angiography can be used to predict the outcome of the operation Note that almost *every patient who benefited from the operation had a*

Table I *Occurrence of collateral circulation and poststenotic dilatation and result of surgery in 28 patients with various degrees of renal artery stenosis*

C=cured I=improved U=unimproved

	Occlusion	Stenosis diameter (mm) <2 0	Stenosis diameter (mm) 2 1–3 0
No of pats	3	23	2
Collateral circulation	3	13	0
Poststenotic dilatation	–	22	1
Result of surgery			
C	3	18	
I		4	1
U		1	1
C	3	10	
I		3	
U			
C		17	
I		4	1
U		1	

stenosis with a diameter of less than 2 mm This corresponds to a reduction of about 80% or more which agrees with experimental studies indicating that this degree of stenosis is required in renal arteries to produce a significant reduction of distal flow and pressure It is therefore not of interest to use as in the study of Erikson et al a 50% reduction of the luminal diameter as a limit

In summary About 80% of patients with proved renovascular hypertension have an abnormal urogram I v urography carried out and interpreted properly is therefore of significant value in the screening of patients with renovascular hypertension

Angiography in renovascular hypertension should be used not only to demonstrate the anatomy of the vascular changes but also to determine their hemodynamic significance The angiography is therefore of great significance in selecting patients for surgery

Sincerely yours

Ingvar Andersson and *Sven Erik Bergentz* Department of Diagnostic Radiology and Department of Surgery Malmö General Hostpial University of Lund Malmö Sweden

Sir

Concerning the letter to the Editor from Andersson and Bergentz we would like to discuss the following points

1) We agree that it is extremely important to define the terms used However we also prefer that readers of one s article note the terms used Thus we discuss a comparison between urography and angiography not in renovascular hypertension but in investigation of renovascular hypertension This is an important difference

We may add that our investigation procedure for diagnosing renovascular hypertension also includes determination of plasma renin activity in the renal veins or their branches This has been done for many years On the other hand this subject was not the purpose of the article

Furthermore there is a possibility of misunderstand the comment by Andersson and Bergentz when they st: It is therefore not of interest to use as in the study Erikson et al a 50% reduction of the luminal diamete a limit We do not regard a certain luminal reductior proof of renovascular hypertension This is provided the results of selective plasma renin measurements fr the renal veins as well as the behaviour of the hypert sion i e the drug efficacy etc We only use the classif tion of luminal reduction common in angiologic diagn tics (cf ref Bergentz et al) to describe the degree stenosis This does not mean that this classificatior etiologically meaningful in fact hemodynamic studies several research groups have shown it not to be

2) The technique of i v urography in our departm includes nephrotomography We have previously use rapid sequence technique but have found it to be of li value and it is now not included in our routine diagno: work

3) Concerning the technique for angiography we w to mention that the catheter used has an outer diamete 2 8 mm It has been used in the same material for m than 15 years (We have not found that this diameter d any harm in renal angiography) A catheter of the sa shape is available in two outer diameters 2 8 mm and mm With the technique used the number of injecti can be decreased and we only use selective studies in kidneys In many cases we use videodensitometry study the renal cortical blood flow

When introducing the catheter into a stenotic artery i very important to have a side hole to avoid total occlusi That is the reason why we have used our catheter seems that the authors of the letter have not underst that point

Sincerely yours

U Erikson A Hemmingsson A Ljungstrom a *H Åberg*

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REVIEW ARTICLE

DNA Repair and Human Disease

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DNA repair mechanisms are of fundamental importance for the protection of the genetic material against structural alterations due to intrinsic metabolic reactions or influences from environmental carcinogens and mutagens

Lately a number of rare inherited human disorders have attracted interest because of tentative or proven defects in DNA repair mechanisms Most well known is xeroderma pigmentosum (XP) previously regarded mainly as a hereditary photodermatosis associated with cutaneous malignancy Today the disease is defined in terms of molecular genetics as a disorder affecting specific cellular DNA repair functions

The importance of this progress is apart from providing insight into the molecular basis of DNA repair that new information has been acquired about the cause of somatic mutations and the possible etiology of neoplastic transformation in man

DNA repair at the molecular and enzymatic level

DNA damage may arise spontaneously or can be induced experimentally by physical chemical and biological agents many of which are natural parts of our environment The enzymatic DNA repair mechanisms of human cells as well as of most eu and prokaryotic cells serve to reconstitute the damaged DNA before DNA replication—*excision repair*—or to restore the original DNA sequence after replication—*post replication repair* (30) Both repair processes involve 1) the recognition of the damaged site and 2) the substitution of a defect DNA sequence with the original one

Normal human cells are capable of repairing different types of DNA damage introduced by ionizing radiation ultraviolet (UV) light and by various chemical mutagens Very likely different enzymes are involved in the recognition and repair of these diverse DNA lesions The repair of DNA damage induced by UV light has been thoroughly studied The UV light gives rise to cyclobutane rings between two adjacent thymine bases on the same DNA strand a thymine dimer Fig 1 shows the four presumed enzymatic steps taking place in normal cells during excision repair of such dimers

The recognition of the dimer initiates a single strand scission of the DNA close to the dimer by a specific endonuclease followed by excision of the defective nucleotide sequence by an exonuclease The single strand interruption is sealed by a polymerase for which the opposite strand serves as the template providing the correct nucleotide sequence Finally the free ends of the DNA molecule are joined by a ligase

Cells which have passed through DNA replication while still carrying DNA damage are thought to contain gaps in the nascent strand opposite the damage The subsequent repair process i e post replication repair reflects gap filling and probably involves short recombinational exchanges between parental and daughter strands ensuring replacement of the damaged sites by the correct DNA sequence

Consequences of reduced or defective DNA repair

Bacteria with deficient DNA repair functions exhibit an increased frequency of spontaneous mutations and enhanced sensitivity to mutagens The association between failure to repair induced DNA damage and an increased frequency of chromoso

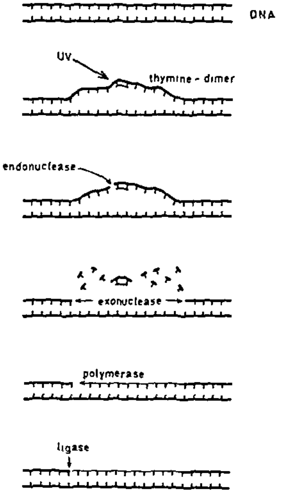


Fig 1 *Presumed mechanism for the repair of UV-induced* thymine dimers in DNA

berrations is well documented in mammalian cells vitro Certain mutations may decrease the re nce of the cell or change its reactions towards ternal carcinogenic factors or may in itself be the crucial event which transforms the cell into malignancy

Another consequence of a deficient DNA repair is cell death During growth or in adult life, an increased rate of cell death affecting tissues with limited proliferative capacity could lead to a broad range of defects including more specific dis turbances such as immunodeficiency neurological abnormalities or to generalized early senescence

Thus some predictable consequences of reduced or defective DNA repair would be increased risk for neoplastic transformation due to somatic muta tions and premature ageing or immunologic im pairment due to an abnormal rate of cell death

The human disorders or conditions which so far have been associated with a reduced DNA repair capacity all display one or several of the presume consequences mentioned

Xeroderma pigmentosum (XP)

XP is a rare autosomal recessive disease with in creased susceptibility to solar radiation Soon afte birth the patients develop erythema edema an blistering Freckles appear on sun exposed areas c the skin which become dry Skin atrophy with in creasing numbers of actinic keratoses hyperkera totic verrucous papules basal and squamous cel carcinomas develop later The incidence of ma lignant melanoma is increased

In some patients the cutaneous lesions are as sociated with neurological abnormalities such a microcephaly with progressive mental deficiency loss of hearing choreoathetosis and ataxia (De Sanctis Cacchione syndrome) The neurologica abnormalities seem to depend on a diffuse loss of neurons

In 1968 Cleaver observed that fibroblasts from patients with XP have a reduced capacity to remove UV induced thymine dimers by excision repair (10) The DNA repair defect was later observed also in other cell populations such as lymphocytes (4) and epidermal cells (17) In several cases the repair o X ray induced DNA damage has been investigated and shown to be normal (32)

More than 60 patients with XP have been re ported DNA repair of UV induced lesions has been analysed and evidence for genetic heterogeneity has been obtained (50) The DNA repair synthesis in different patients has been variable ranging from 2 to 70% of that of normal cells The technique of somatic cell fusion has made it possible to study genetic complementation in XP When fibroblasts are mixed in the presence of killed Sendai virus the cell membranes coalesce the cytoplasm becomes intermingled and multinucleated heterokaryons are formed (Fig 2) Heterokaryons formed between fibroblasts from patients with XP and normal fibroblasts show as expected a restoration of the DNA repair synthesis However also cells from different XP patients may complement each other Thus heterokaryons formed by fusion of repair de ficient fibroblasts from certain groups of XP pa tients display an almost normal capacity to repair UV induced DNA damage So far five complemen-

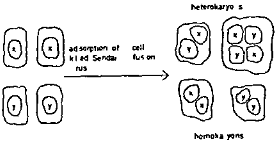


Fig 2 Schematic presentation of the technique of somatic cell fusion

tation groups have been distinguished (34 59) Therefore it seems possible that at least five different genetic loci are involved Moreover some patients with the typical features of XP but with normal excision repair have been observed (XP variants) It was recently suggested that cells from these patients are deficient in the post replication repair (38)

Syndromes associated with chromosomal instability

Several inherited human disorders with a characteristic chromosome instability in peripheral lymphocytes have been suspected on a theoretical basis or indicated by experimental results to be associated with various types of DNA repair defects (18 26) These disorders include Fanconi's anemia ataxia telangiectasia and Bloom s syndrome

Patients with *Fanconi's anemia* (FA) are frequently small at birth and exhibit a stunted post natal growth Mental retardation of a moderate degree may be present The patients often have cardiac kidney and skeletal anomalies Brownish skin pigmentation is a characteristic finding but there is no increased sun sensitivity The main symptoms are bleedings due to pancytopenia predominantly affecting the trombocyte forming cells

There is a strong predisposition for leukemia and certain rare forms of cancer (18) Peripheral lymphocytes display an increased frequency of chromosome aberrations (6 33) and there is an increased incidence of chromosome breaks after treatment of cells in vitro with various alkylating and DNA cross linking agents (2 51 53)

Evidence suggesting a defective DNA repair in FA has been presented Poon et al (46) demonstrated a reduced capacity for excision of UV induced thymine dimers in fibroblasts from patients with FA although the UV induced repair synthesis seemed to be normal Sasaki (51) presented recently some evidence suggesting that cells from patients with FA may be impaired in the endonucleolytic function of the initial step in the repair of cross links between the DNA strands

Ataxia telangiectasia (AT) is also an autosomal recessive disorder Constant symptoms are a progressive cerebellar ataxia and oculocutaneous telangiectasia The children seem normal at birth Symptoms usually appear at the age of 12 to months and progress into invalidity at the time of adolescence Autopsy in some cases has revealed a uniform atrophy of the cerebellar cortex and demyelination of the posterior columns and spino cerebellar tracts of the spinal cord The telangiectases first appear in the bulbar conjunctivae but later develop in other areas of the face as well and in non exposed areas of the trunk and extremities The syndrome is usually associated with a severe immunodeficiency

AT is associated with an increased incidence of malignancy particularly of lymphoid tissues (18 45) and there is an increased frequency of chromosomal aberrations in peripheral lymphocytes and fibroblasts (11 42)

Patients with AT may display an extreme radiosensitivity (56) Severe complications in some cases culminating in premature death have been described after conventional tumor radiotherapy Radiosensitivity has also been observed at the chromosomal level in vitro in X irradiated cells from patients with AT Recent evidence indicates that this radiosensitivity is related to a defective excision repair of DNA damage caused by ionizing irradiation (44 57)

The cardinal features of *Bloom s syndrome* (BS) are pre and postnatal growth inhibition sun-sensitive telangiectatic erythema and characteristic chromosome rearrangements in peripheral lymphocytes The patients often have a long and narrow head (dolichocephaly) with a prominent nose relatively hypoplastic malar areas and retracted mandible The mental development is normal

Malignancy is highly frequent among subjects with BS In a follow up study by German (personal communication 19) 10 out of 63 cases have developed malignant disorders including cancers sarcomas and leukemias It is worth noting that all subjects followed beyond the age of 30 have veloped malignancy

In a large percentage of peripheral lymphocytes there are specific cytogenetic abnormalities which are of diagnostic importance in BS An increased frequency of sister chromatid exchanges is another characteristic finding (7) Evidence for a specific DNA repair defect in BS is lacking UV exposed cells from patients with BS display a reduced survival and other indirect observations suggest that the affected enzymatic reaction rather than being absent is slower than normal (25)

Syndromes associated with premature ageing

The association between DNA repair and premature ageing is mainly based on theoretical considerations predicting an age related accumulation of mutations in somatic cells Defective or reduced DNA repair is thought to increase the rate of somatic mutations resulting in impaired cell functions cell death and early senescence

The autosomal recessive disorders described under this heading include Cockayne s syndrome progeria and Werner s syndrome The children affected with these disorders appear normal at birth

In *Cockayne s syndrome* growth and motor mental development becomes abnormal in the second year of life ending with dwarfism retinal degeneration cataract microcephaly mental retardation skeletal deformities and a typical senile face There is a characteristic photosensitive skin rash which heals when protected from daylight

Life span is shortened and there is no report of an increased incidence of malignancy or chromosome aberrations Recently Chu and co-workers (personal communication) have made preliminary observations of a reduced colony forming ability and a decreased DNA repair synthesis after UV exposure of cultured fibroblasts from seven unrelated patients with Cockayne s syndrome The repair defect may be related to a decreased DNA polymerase activity

Progeria causes growth retardation senile skin changes and loss of hair in infancy or early childhood Intelligence is usually normal Cardiovascular disease develops between 5 and 15 years of age and death usually soon follows often from myocardial infarction No increase in malignancy or chromosome aberrations have been reported

Skin fibroblasts from progeria patients show a diminished growth capacity and increased heat lability of some enzymes in vitro (20)

Some still disputed results (48) presented by Epstein et al (15 16) indicate that progeria fibroblasts in vitro have a decreased rate of rejoining of γ ray induced DNA strand breaks Thus a defect in DNA repair specific for ionizing radiation may be associated with this disease

In *Werner s syndrome* the features of premature ageing first become apparent during puberty Growth arrest resulting in short stature development of cataract premature hair graying and balding early arteriosclerosis osteoporosis scleroderma and skin ulcers diabetes mellitus and hypogonadism are characteristic clinical symptoms

About 10% of the patients are affected by neoplastic disease (14) Fibroblasts derived from skin or subcutaneous biopsies display a reduced growth potential in vitro (14) No gross chromosomal abnormalities seem to occur in vivo or in vitro Various enzyme defects have been reported in cultured fibroblasts from patients with Werner s syndrome (21 22 23) To our knowledge there are no studies of DNA repair in this condition

Other conditions associated with an increased cancer incidence

Down s syndrome (DS) (mongolism) is associated with an increased incidence of leukemia (28 31 55) The enchanced sensitivity to infectious diseases (24) and the high incidence of Au antigenicity (3) in DS suggest a basic immunologic deficiency

Cells from subjects with DS exhibit an increased frequency of chromosome aberrations after X ray irradiation (29 52) treatment with chemical mutagens (54) or viral infections in vitro (27 58) Cells from subjects with DS furthermore display a slower proliferation rate in vitro than normal cells and exhibit a reduced DNA polymerase activity (1) A slightly reduced UV induced DNA repair synthesis has been reported in lymphocytes (35) and fibroblasts (Bayreuther personal communication) from subjects with DS The significance of these observations in relation to induction of malignancy sensitivity to chromosome breakage and viral infection or the suggested immunological deficiency is at present not clear It should be noted however that DNA damage induced by many chemical carcinogens and mutagens is probably repaired by the same mechanisms that remove UV induced DNA damage

Actinic keratosis is a premalignant lesion of the epidermis Extensive exposure of the skin to sunlight is generally considered as an important

factor in the etiology of this condition At least 15% of the patients who are left untreated with these lesions develop squamous cell carcinomas

The clear association between actinic keratosis and UV exposure suggests that subjects acquiring these lesions might have a repair capacity lower than average In a group of 10 subjects with multiple actinic keratoses the UV induced DNA repair synthesis was about 30% lower than in healthy control subjects of the same age (37) Thus a reduced *UV induced DNA repair synthesis may be an additional* important factor predisposing for actinic keratosis and possibly actinic malignancy

Epidermodysplasia verruciformis is a rare dermatosis associated with multiple skin cancers A reduced UV induced DNA repair synthesis in peripheral leukocytes from a patient suffering from this disease is reported in a separate article in this issue

Ageing and malignancy are closely linked in the general population It has also been found that malignant disease induced by X ray exposure is more frequent in higher age groups (13) An increased frequency of structural chromosome aberrations single strand DNA breaks and single stranded DNA regions have been described in older cells of mammals and man (8 39 47 60) Such age related modifications of the genetic material have been regarded as an essential factor in the ageing process (5 12 41), and could obviously reflect age-dependent alterations in DNA repair mechanisms Attempts to demonstrate deficiencies of the DNA repair mechanisms of ageing cells in vitro have given inconclusive results (9 40 43)

The normal ageing is associated with many cellular changes It is therefore not totally unexpected to find a moderate but significant reduction in the capacity for DNA repair synthesis in higher ages In a study of 48 healthy subjects between 13–94 years of age the UV induced DNA repair synthesis was shown to correlate inversely with increasing age (36 49)

The additional observation (49) of a great variation in the capacity for DNA repair synthesis among healthy subjects of similar age is of considerable interest since it suggests that the resistance against external carcinogenic and mutagenic influence may be related to a similar individual variation

Concluding remarks

A number of human disorders or conditions which are associated with impairment of DNA repair functions have been presented Most of these disorders are rare and of limited importance in general preventive and clinical medicine However they may set examples for a new and fruitful insight into basic molecular and cellular processes of importance for growth and development and for the resistance and susceptibility of man towards neoplastic transformation

Interest in DNA repair is expanding also within other fields of research dealing with the genetic hazards of environmental *mutagens and carcinogens* It is likely that forthcoming studies on human disorders which exhibit cancer susceptibility chromosomal instability increased sensitivity to radiation immunological deficiency impaired neurological development or premature ageing will provide fundamental information for the understanding of DNA repair mechanism and their importance in human medicine

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Oncology—a part of internal medicine

In many countries oncology has been a special subject with special journals chairs and departments The Scandinavian countries have been rather conservative in this respect and the Acta Radiologica with its different sections all covered by the title radiologica is an example of this situation It seems natural that the hospital departments that have been called radiotherapy will in future be transformed into departments of oncology This means that they will come much nearer internal medicine and there is no doubt about the fact that chemotherapy endocrinology immunology will become of increasing importance in the care of the cancer patient or in medical cancer research

The Acta Medica Scandinavica is very much aware of this trend and we shall welcome oncological papers within different fields if they are of general medical interest and increase our understanding of fundamental medical and biological problems This number contains an original paper from the fields of dermatology radiation molecular biology and their contact with the problems of oncogenesis The same authors have also written a review article of their field We hope to be able to bring several papers of this kind during the next year 1977 The editors would like to invite authors in the field to bring communications and editorials

During 1976 the Swedish Medical Society has started a new section for oncology It is hoped that this will become a forum of debate between all specialties actively interested in care of the patient with neoplastic disease *and* also basic scientists working on fundamental problems in cancer research The future will need both well educated specialist but also doctors with a broad understanding of cancer problems

Jan G Waldenström

Acta Med Scand 200 441–446 1976

A Case Report Including EM and DNA Repair Investigations in a Dermatosis Associated with Multiple Skin Cancers Epidermodysplasia Verruciformis

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ABSTRACT This report describes the clinical histological and electron microscopic observations in a 51 year-old male with epidermodysplasia verruciformis (EV) Cells with early signs of malignant transformation were found closely connected with virus infected epidermal regions Skin cancers appeared initially on sun-exposed areas, such as the face and ear lobes The UV induced DNA repair synthesis was therefore studied, utilizing peripheral leukocytes The patient had 40% lower UV induced DNA repair synthesis than the mean of nine healthy subjects of the same age These results suggest that a decrease in UV induced DNA repair synthesis in combination with a possibly oncogenic viral infection may enhance the disposition for somatic mutations and malignant transformation in patients with EV

Epidermodysplasia verruciformis (EV) is a dermatosis in which plane papules appear in teenage and disseminate to a great extent in early adulthood (8) Common wart virus has been identified in such lesions by electron microscopic and virological methods (16) Early development of skin malignancies occurs in EV and the viral infection has been incriminated as the oncogenic factor (8) Signs of the viral infection are lost however in the transformed malignant cells (8 16) and the presence of the viral genome in such cells has not been shown so far (16)

It seems possible that other factors might be effective as well for the malignant transformation in EV Light induced damage to the skin is known to produce skin cancers at an early age in patients with the autosomal recessive disease xeroderma pigmentosum Mammalian cells have a capability to repair certain types of DNA damage enzymatically (7 15) Patients with xeroderma pigmentosum are affected by a gene mutation which seriously impairs this function (2 14) Thus in these patients the defective repair of UV damage to DNA is associated with an increased risk of neoplastic transformation

This paper presents a study of UV induced DNA repair synthesis in a patient with EV in addition to histological and electron microscopic observations of his skin lesions The results indicate that the viral infection in combination with a decreased capacity to repair UV induced DNA damage might be pathogenetic factors in the development of malignancy in EV

CASE REPORT

The patient is a 51 year-old man who used to be a metal worker and for the last 20 years has served as a cook He was unaware of any skin disease in his family In his thirties he was treated for gastritis and ventricular ulcers and in 1964 for appendicitis In 1969 pulmonary tuberculosis was diagnosed After treatment for one year the signs of the disease had disappeared

In his teens plane papules appeared first on the hands and later on the trunk and extremities (Figs 1–3) In 1953 Darier s disease was suggested on indicative traits in a biopsy specimen from a pityriasiform papulous lesion In 1965 when the patient was 41 years old the first basal cell carcinoma appeared in his face Since then there have been multiple manifestations of neoplastic cutaneous disease in his face basal cell carcinomas were excised 14

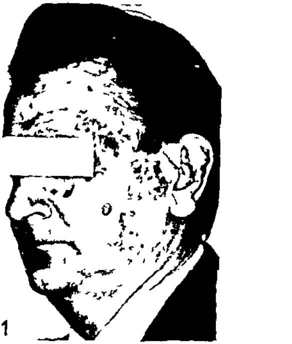


Fig 1 The patient when 43 years old Several lesions of Bowen s disease basal and squamous cell carcinomas are present which required multiple excisions and transplanta tions

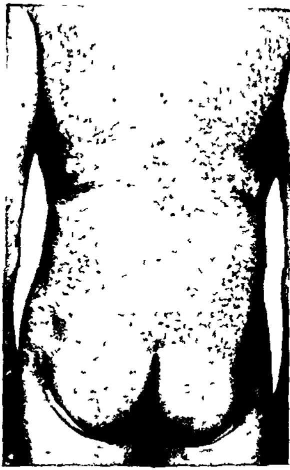


Fig 2 Lesions of epidermodysplasia verruciformis The patient is 51 years old

times surgical treatment was given 7 times for lesions of Bowen s disease non invasive squamous cell carcinomas were excised 8 times and on one occasion (1971) an inva sive squamous cell carcinoma located on the right cheek was treated with both radiotherapy and surgery In 1975 the patient was admitted to the Department of Dermatolo gy Karolinska Hospital for treatment of multiple lesions of actinic keratosis and Bowen s disease

On physical examination the patient displayed many eratotic and ulcerated lesions on the face and ear lobes) cicatricial atrophic area around the left eye was served and an induration was noted in a transplant on the right cheek On the buccal aspect of this area there was a hard ulcerated papillomatous lesion The extremi ties and the trunk were covered with slightly elevated scaling papules—some of which appeared as plane warts others resembled pityriasis versicolor—and several kera totic lesions indicating a possible Bowenoid transforma tion (Figs 3 4)

Laboratory investigations ESR was 37 mm/h Hb blood cell counts liver enzymes urinalyses and serum immunoglobulins showed values within the normal range Intracutaneous tests with *PPD* candidin and mumps showed positive reactions after 48–72 h The patient was not allergic to dinitrochlorobenzene and could be sen sitized to this compound after challenge according to Foussereau et al (6) These tests indicated a normal de layed immunologic response A KOH study on scrapings from the scaling lesions on the trunk (Fig 3) revealed few filaments consistent with Pityrosporon furfur



Fig 3 Close ups of the lesions from (*a*) the back and (*b*) the back of the hand The arrowed lesions displayed Bow en s disease on histological examination Similar lesions to those of the others on the back showed Bowenoid actinic keratosis In some lesions scales provided Pityrosporon furfur The left hand papule on the back of the hand is consistent with a plane wart

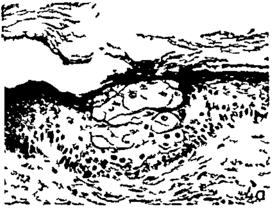
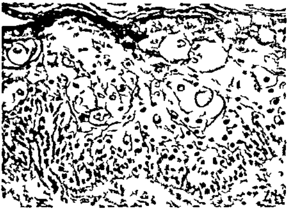
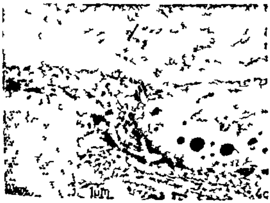


Fig 4 Papule from the dorsal aspect of the hand clinically judged as a plane wart as shown in Fig 3*b*

(*a*) An area of clear cells with scattered keratohyalin granules in the subcorneal cells Pyknotic and giant clear cells could be seen in the basal part of the epidermis This alteration is characteristic of a plane wart in epidermodysplasia verruciformis H & E ×370



(*b*) An adjacent portion of the same epidermal section as in *a* Some of the characteristics shown in *a* are present also here In the spinosum layers epithelial atypia is shown consistent with a transformation into Bowen's disease H & E ×79?



(*c*) EM micrograph of subcorneal clear cells containing round keratohyalin granules without tonofilaments The cell nucleus is loaded with virus particles (×6336) The insert shows the virus particles at higher degree of magnification (×35 442)

Histologic examination Punch biopsies from papules on the hand showed histological features common to EV (Fig 4*a*) Moreover punch biopsies from several keratotic and ulcerated skin lesions revealed actinic keratoses and lesions of Bowen's disease A biopsy from the ulcerated tumour on the right buccal mucosa displayed a low differentiated invasive squamous cell carcinoma

The patient received topical treatment with 5% 5-fluoro-uracil ointment on the face and trunk in seances To speed up the reaction to 5-fluoro-uracil during treatment of the trunk this substance was combined with 0.05% tretinoin ointment twice a day Reticulated eroded areas developed after 1 3 weeks treatment and healed within another week or two after the treatment had been



(*d*) EM micrograph of spinosum clear cells containing many organelles and several nucleoli but lacking tonofilaments Intercellular attachments and desmosomes are found as in the normal keratinocytes ×4422

Table I *Effect of hydroxyurea (HU) on the incorporation of ³H thymidine into non irradiated leukocytes from the patient and nine controls*

	No of experiments	Incorporation of ³H thymidine (cpm/10^6 cells ± S D)	
		No HU	10^{-2}M HU
Patient	5	840±388	95±27
Controls	9	1 215±578	75±15

discontinued Combined treatment with radiotherapy and surgery was planned for the buccal squamous cell carcinoma but had to be dropped when pulmonary tumours appeared before the surgery could be performed Palliative cyclophosphamide treatment has been instituted instead

MATERIAL AND METHODS

Histological and electron microscopic investigations Biopsy specimens were taken from the back of the hands one from a plane circumscribed papule and another from a scaling papule with an indistinct border (Fig 3*b*) they were processed for routine histology and stained with hematoxylin and eosin (H & E) A similar pair of lesions on the hand were excised for electron microscopy Part of the lesions was fixed in formalin embedded in paraffin sectioned and stained in H & E The remaining part was fixed in 4% glutaraldehyde in 0 1 M phosphate buffer pH 7 2 overnight at 4 C rinsed in the buffer solution post fixed for 1 h in 2% osmium tetrahydroxide rinsed in 0 1 M veronal buffer pH 7 2 for 30 min dehydrated and embedded in Spurr's resin (Taab Laboratories Reading England) The sections were stained with 4% uranyl acetate for 1 h and 4% lead acetate for 5 min Micrographs were taken with a Philips EM 300

DNA repair investigation The UV induced DNA repair thesis was measured according to Evans and Norman) with some modifications (10) Peripheral leukocytes re obtained from 10–20 ml of freshly collected heparinized venous blood Five different samples were drawn on separate occasions from the patient and compared with samples from nine apparently healthy subjects 41–61 years old (mean 52 4 S D =6 6)

White cells were washed in phosphate buffered saline and exposed to UV light (254 nm) in four doses of 3 2–19 2 J/m² After irradiation the cells were incubated in 1 ml Parker 199 medium (Flow Laboratories) supplemented with 25% of fetal calf serum 125 µg streptomycin and 125 IU of bensyl penicillin Hydroxyurea (HU) to a final concentration of 10^{-2} M was added to five of the tubes including 1 unexposed control sample Two tubes comprising one unexposed sample and one exposed to 9 6 J/m² did not receive any HU

After preincubation for 30 min at 37 C ³H thymidine 5 Ci/mM 1 mCi/ml (The Radiochemical Centre Amersham) was added to a final concentration of 10 µCi/ml The incubation continued for 2 h and was interrupted by addition of 1 ml cold 10% trichloroacetic acid (TCA) Free nucleotides were extracted with TCA and analyzed for radioactivity in a Packard liquid scintillation spectrometer at an efficiency of about 30% and a back ground of 20 cpm

RESULTS

Histological and electron microscopic study of plane papules

The lesions suggestive of a plane wart showed a patchy or continuous abundance of clear cells near the corneal layer and extending in some areas down to the suprabasal layer (Fig 4*a*) The subcorneal clear cells contained many abnormal keratohyalin granules in the cytoplasm (Fig 4*a–c*) Tonofilaments were sparse and many enlarged mitochondria exhibited cristolysis (Fig 4*c*) The cytoplasm contained a dense granular matter The nucleus had a convoluted membrane and contained large amounts of viral inclusions Adjacent subcorneal keratinocytes were normal in appearance In one part of the plane papule the subcorneal epidermis displayed the viral inclusion pattern present in Fig 4*a* combined with Bowenoid traits in the basal parts of the epidermis (Fig 4*b*) EM micrographs from such areas displayed cells with normal desmosomes sparse in tonofilaments rich in cytoplasmatic organelles with cristolysis in mitochondria and with a nucleus containing two or more nucleoli Viral inclusions were not present (Fig 4*d*)

Study of UV induced DNA repair synthesis in peripheral leukocytes

The UV induced DNA repair synthesis was measured after treatment of the cells with HU which inhibits the replicative DNA synthesis but does not affect the repair synthesis (4) The effect of HU on non irradiated leukocytes is shown in Table I The HU depressed the replicative DNA synthesis to about the same level in leukocytes from the patient and the controls

The UV induced DNA repair synthesis was studied after exposure with different UV doses The incorporation values are presented in Table II At all UV doses given the patient showed a 40% reduction of the UV induced DNA repair synthesis compared with mean values for the controls The individual values for the controls and the repeated measurements in the patient are shown in Fig 5 A considerable variation in UV induced DNA repair

Table II *UV induced DNA repair synthesis in leukocytes from the patient and nine controls*

	No of experiments	Incorporation of ^{3}H thymidine (cpm/10^6 leukocytes±S D) at different UV doses (J/m^2)			
		3 2	6 4	9 6	19 2
Patient	5	426±197	562±106	601±162	599±129
Controls	9	715±221	881±272	950±261	1 023±310

synthesis is present in the controls as observed previously (13) However only one control subject has a UV induced DNA repair synthesis which is below that of the patient

DISCUSSION

EV is a rare disorder which predisposes for cutaneous malignancy Our patient had multiple actinic keratoses many lesions of Bowen s disease and of basal cell carcinomas and invasive squamous cell carcinomas The neoplastic diseases developed initially on sun-exposed areas cheeks ear lobes and lateral rims of the orbita Most lesions recorded as Bowen s disease on the trunk were located on the sternal region A few lesions were seen on the buttock and on the legs Familial aggregation is often found in EV (8 12) but there is no proof of mendelian inheritance The family history of this patient is unknown

Since cutaneous malignancy in the patient was mainly associated with sun exposed areas an increased sensitivity to UV light might be expected In xeroderma pigmentosum there is a close association between increased UV sensitivity the appearance of tumours on sun exposed skin and a defective DNA repair Cultivated fibroblasts (2) as well as peripheral lymphocytes (1) from these patients display a reduced capacity to repair UV induced DNA lesions

According to present concepts repair of the thymidine dimer the principal UV damage to DNA involves four enzymatic steps the DNA strand is incised by an endonuclease close to the damaged site after which an exonuclease digests the DNA around the lesion removing about 100 nucleotides Then a polymerase synthesizes a new strand of DNA complementary to the unaffected DNA strand and the open ends of the molecule are connected by a ligase (7) Recent studies using cell fusion have revealed five complementation groups among xeroderma pigmentosum patients having a defect in the excision repair of UV induced DNA lesions (9 14) Thus different enzymatic defects may cause similar clinical symptoms in the case of xeroderma pigmentosum

Measurements of UV induced ^{3}H thymidine incorporation as in this study only reflect the polymerase activity of the DNA repair process However since the incision step as well as the excision step of the tentative DNA repair mechanisms are required before the polymerase starts to act it is very likely that the present technique gives an estimate of the sum of the first three repair steps

In most patients with xeroderma pigmentosum the UV induced DNA repair synthesis is reduced by over 50% of control values (9 14) In a group of patients with actinic keratosis studied by the same

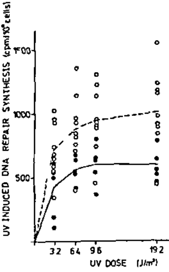


Fig 5 UV induced DNA repair synthesis in peripheral leukocytes from the patient with epidermodysplasia verruciformis (●) and nine controls (O) The cells were irradiated with different UV doses preincubated for 30 min with 10^{-2}M HU and subsequently incubated for 2 h in the presence of 10 μCi/ml ^{3}H thymidine and HU The background of HU treated non irradiated cells is subtracted

technique as in the present report the UV induced DNA repair synthesis was decreased to about 70% of that found in healthy age matched control subjects (11 13) The patient with EV was found to have about 60% capacity for UV induced DNA repair synthesis compared with control subjects of the same age group Another DNA repair study in a patient with EV did not reveal any difference from the controls (D Bootsma, personal communication)

We have previously observed (13) that there is a considerable variation among normal healthy subjects with regard to the UV induced DNA repair synthesis Thus in addition to the inherited defect in xeroderma pigmentosum there probably exist individuals with a UV induced DNA repair capacity which is within the normal range but considerably lower than average Such individuals may display an increased risk of developing malignancy of the skin after extensive solar radiation as with actinic keratosis or when other predisposing factors are present as we believe may be the case in EV

The histological and electron microscopic observations in the patient with EV stress the close relation between the virus infected cell nests and the dysplasia of the epidermal cells It is probable that malignant cells can arise in the virus infected areas as is suggested from the present figures The oncogenic capacity of the viral infection is not shown by this result but it is probably one etiologic factor in the development of the skin cancers in EV An additional factor is indicated by our results on the decreased DNA repair synthesis in the patient with EV and by previous studies indicating that a decreased UV induced DNA repair capacity may play an important etiological role in actinic malignancy (11 13) We suggest that in EV the predisposition to acquire a possibly oncogenic virus infection in combination with a decreased capacity for UV induced DNA repair synthesis may enhance somatic mutations and malignant transformation

ACKNOWLEDGEMENTS

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Treatment of Malignant Metastatic Pancreatic Insulinoma with Streptozotocin

Review of 21 Cases Described in Detail in the Literature and Report of Complete Remission of a New Case

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BSTRACT The possibilities of treating different pes of pancreatic islet cell cancer with the active ug streptozotocin have been studied in reports of patients published during the last 8 years The usual features of the symptomatology, dosage and jection methods, side-effects after different infusion hedules and complications of streptozotocin erapy, are compared A new case with pancreatic rcinoma and liver metastases was treated with a tal dose of 14 g streptozotocin, divided into 7 injec ons with an increasing interval between them This tient underwent a marked amelioration of the sease, without serious side-effects of the streptozo- cin treatment She has been apparently well for the ! months which have now elapsed since the begin ng of treatment This seems to be one of the longest mission periods yet published in the insulinoma erature

sulin producing malignant β-cell tumours of the increas are not common The early diagnosis of fferent islet cell tumours is often difficult Arnesjo t al (1) recently reported 31 insulinoma cases 1 25 of their patients more than one year had lapsed between the onset of the symptoms and the iagnosis and in a further 4 patients the tumour mained undetected for more than 10 years In 1 extensive American review (9) of 398 cases of ancreatic tumours it was concluded that without rly effective surgical extirpation of the tumour nd/or pharmacological treatment the malignant rcinoma patients survived on an average less than ne year

For reducing the high and dangerous serum sulin values several types of pharmacologically active substances have been tested These include insulin reducing agents such as corticosteroids glucagon human growth hormone and diazoxide and anti-cancer agents such as tubercidin (7-deaza adenosine) 5 fluorouracil alloxan L asparaginase nitrogen mustard and streptozotocin (14 15)

The most active agent for achieving a permanent remission of pancreatic insulinoma tumours is streptozotocin an antibiotic isolated from cultures of Streptomyces acromogenes Chemically it is a compound of a well known alkylating agent 1 methyl 1 nitrosourea and glucose (8) Biochemically it is an inhibitor of the synthesis of pyridine nucleotides NAD and NADH The first streptozotocin treated case was reported in 1968 (11)

The cancer therapy evaluation branch of the National Cancer Institute (USA) has recorded 52 cases of streptozotocin treated islet cell carcinomas In this series good remission results were obtained in about 10 cases (14) The symptomatology of insulinomas varies considerably and the administration technique dosage and total amounts of injected streptozotocin differ widely as do the results of treatment The aim of the present paper is therefore to summarize 21 detailed case reports of streptozotocin treatment from the literature In addition a new successfully treated case of malignant insulinoma is described

REVIEW OF STREPTOZOTOCIN TREATED PUBLISHED MATERIAL

The 21 cases described in the literature were treated between 1968 and 1975 All had a malignant islet cell car

... clinical symptoms streptozotocin administration schedules toxic effects and the duration of remissions in 21 ... er metastatic insulinoma patients published in detail during the last 8 years

No of treated pats	Age (y)	Sex	Special clinical signs and symptoms in addition to conventional symptoms	Streptozotocin: Administration technique and time	Dose/d (g)	Total amount injected (g)	Side effects	Length of remission during clinical observation time	Reference no
1	59	♀	Diarrhoea increased serum gastrin and glucagon	I v infusion 15 min	1 5–4	8 5		8 mo	11
1	55	♂	High elevation alc phosph SGOT SGPT	I v injection	1 5–3	4 5		4 mo	2
2	54	♂	Gastric peptic ulcer serum gastrin elevation	Aorta catheterization to celiac axis	1 (10 d)	10	Glucose cysteine glutamine alanine in urine	4 mo but certain kidney damage	13
	?						Acute tubular necrosis	† 7th day*	
	50	♂		I v and i a celiac axis	2–4	8 i v 15 i a	Liver enzymes rose protein and amino-aciduria	6 mo	18
	68	♀	Metastases in spleen omentum mesenterial tissue pelvic organs	I v infusion 30 min	1 7–3 4/week	11 2	Proteinuria	5 mo	3
	70	♂							
	60	♂		I v infusion 60 min	2–4 (2 week intervals)	12 5	Anorexia glycosuria	3 mo	19
				I a celiac axis	3 8–17	29 8	Ileus SGOT SGPT alk phosph elevated	No remission † 28 d	17
	25	♀	Multiple endocrine adenomatosis obesity virilization	I a celiac axis	2–9	36	Fever alk phosph SGOT SGPT elev thrombo-leucocytopenia ren tub acido...	7 mo	

			phagia hepato megalia	15 min			cramp diarrhoea moderate creatinine elevation		6
1	61	♀	Gastric hyper secretion increased serum gastrin duod ulcer	I v infusion 20 min	3–6	13 5	Polyuria polydipsia	4 mo then † perfor duod ulcer	10
1	50	♀	Weight loss spleen enlarge ment	I v cava catheter 4–6-day intervals	1 5	6	–	Unpublished length of amelioration	12
2	48	♀	Duod ulcer cachexia	I v infusion once/mo	3 (per 300 ml water)	9		15 mo	5
	36	♀	Duod ulcer severe derma titis			12		13 mo †	
1	58	♀	Mild diabetes 3 y before tumor mani festation	Slow i v injection	1 5–6 (per week increa sing dose)	18 (per 42 days)	No clini cal ame lioration	After 45 d † paralysed ileus 4 lit ascites total necrosis of pancreas	6
5	57	♀	Varying hepatic complications	I v infusion five-day courses	1 5–7 5 (per m² for 5 d)	13–16	SGOT ele vation nephrotox icity protein uria tubu lar acido-sis glycos uria	12 mo	15
	No effects in 4 cases							No remission in 4 patients 2 patients † after 3 weeks	
1	62	♀	Abdominal pains watery diarrhoea weight loss SGOT elevated	I v	1 5–2 (per week)	7 5	–	15 mo	7

Ad mortem after infusion of 5 fluorouracil into celiac axis

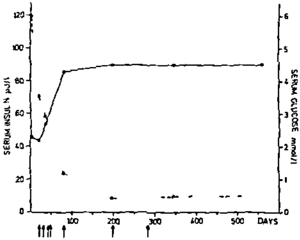


Fig 1 Serum insulin (●) and serum glucose (O) values before during and after the streptozotocin injections Each arrow represents a single i v injection of 2 g streptozotocin After the 9th month no more streptozotocin was given

cinoma with liver metastases They all exhibited the usual clinical symptoms and signs such as severe hypoglycemia very high serum insulin values with attacks of unconsciousness dizziness and various degrees of malignancy The age special and unusual clinical signs and symptoms administration techniques and dosages side-effects and the lengths of remission periods are summarized in Table I Most patients were 50–65 years old (mean 56) In addition to the more common symptoms and signs the *following features were noted high serum gastrin and* glucagon values (10 11) pathological liver enzyme activities (2 7 16) gastric or duodenal peptic ulcer (5 10 13) watery diarrhoea (7 11) multiple endocrine adenomatosis in a 6-year-old child This patient developed an active insulinoma with a tumour severe obesity and virilization (17) Diabetes mellitus was the activating actor in one case 3 years before the development of the ignant tumour (6)

The mode of streptozotocin administration differed widely In some patients the drug was given fairly rapidly i v (duration of injections 5–20 min) in others more slowly (20 min–3 h) In one case the drug was injected into the vena cava via a catheter and in several cases direct intra aortic injection was given via a catheter in the celiac axis The daily dosage varied between 1 and 17 g The total amount of streptozotocin administered varied between 4 and 30 g The duration and length of remission of the insulinomas and metastases also varied considerably In very advanced carcinoma cases with severe malignancy no remission was observed The average length of remission among all the 21 cases was about 7 months

In addition to the frequent side-effects of streptozotocin injections such as nausea vomiting and diarrhoea a *serious complication in 10 patients was a varying degree* of acute tubular necrosis In several cases this renal damage was however reversible

There were various causes of death Death was prob bly accelerated by (*a*) additional infusion of 5 fluorourac (5 13) (*b*) high total doses of streptozotocin (18–30 g) very frequent large amounts of the drug (6 13 15 17 (*c*) perforation of a duodenal ulcer (10) finally (*d*) hig doses in 2 cases (13 5–36 g) resulted in severe diabet insipidus (10 17)

CASE REPORT

Our patient is a 55 year-old woman who has previousl worked in a nursing institution She is the 15th child a family of 16 brothers and sisters Neither diabetes nc any other endocrinological disorder was found in an other member of the family At 47 years of age hyster ectomy was performed because of severe prolonge uterine bleeding In the last year before admission to ou department her body weight had increased 10 kg Durin the final month the patient had drunk several liters c sweet sugar rich fluids daily and nightly The usu insulinoma symptoms such as tremor sweating dizz ness periods of unconsciousness and headache devel oped rapidly The smallest muscular exertion elicite these symptoms especially in the morning In the prev ous year she had also suffered from abdominal pain cramps and heartburn In March 1974 she was admitte to a provincial hospital in a state of coma Between Marc and Nov 1974 the treatment consisted mainly of large i v glucose infusions to counteract her high serum insuli values She came to our department in Nov 1974 wit very low serum glucose concentrations and extremel high serum insulin values It was necessary to infuse he continuously i v with glucose and in addition she dran more than one liter of a glucose solution each night

Investigations

Peroral glucose tolerance tests showed highly increase serum insulin values (about 160 μU/l) Selective coeliaco graphy revealed a solid tumour (1×1 cm) in the pan creatic tail In addition several metastases the size of table tennis ball were present in the liver A liver scan also revealed multiple metastases distributed in variou parts of the liver One metastatic hepatic nodule wa punctured and the biopsy revealed cellular polymorphism and polychromasia Histologically the tumour was highly differentiated β-cell carcinoma Other investiga tions i e chest X-ray haematological data liver enzyme values and the kidney function revealed no abnorma lities

Streptozotocin treatment was started on Nov 17 1974 with an i v dose of 2 g infused during 5 min The dose schedule and results of the treatment are presented in Fig 1 Thus four doses of 2 g were given at 2 week intervals Each infusion was followed by nausea and vomiting for about 24 hours As can be seen in Fig 1 the serum insulin decreased rapidly and the serum glucose values became normal relatively soon To obtain a longer remission we decided to give more streptozotocin 2 g were injected in the middle of Jan 1975 and the last doses later in May and Aug 1975 A further coeliacography half a year after the first streptozotocin dose revealed that the liver metastases were considerably smaller and

...at the large primary tumour in the tail had disappeared. Several liver biopsies failed to show any cancer cells after treatment only normal liver tissue was found. The patient was followed up in our department through short hospitalization periods until Sept 1976 and the serum glucose and insulin values as well as other laboratory data were always normal. The patient seems to be completely recovered clinically and never has any symptoms. She is working hard as a nurse and at least three times a week she runs long distances in the forest. Thus the successful remission time after the first streptozotocin dose is now 22 months.

DISCUSSION

Several types of pharmacological agents and anti-carcinoma substances have proved to be of use in the amelioration of different types of insulin producing β cell carcinomas, especially those with liver metastases. Most hormone preparations gave no clinical benefit. Only hydrochlorothiazide and diazoxide were tested frequently but no real remissions of the tumours were reported. Moreover hydrochlorothiazide produced side-effects such as hypokalaemia, metabolic alkalosis, dehydration and hyperglycaemia. The side effects of diazoxide such as sodium and water retention, oedema, heart palpitations and haematological changes were frequently observed.

Streptozotocin, a potent inhibitor of insulin synthesis and an active anti-cancer agent, is the only drug used frequently in islet cell cancer cases which has resulted in rather good remission (Table I).

After studying the 21 cases reviewed here we suggest the following criteria for obtaining good curative effects of streptozotocin. 1) The treatment must be started immediately after early diagnosis before widely dispersed metastases have time to develop. 2) Intra-arterial administration to the coeliac axis via the aorta must be avoided since part of the injected drug reaches the renal arteries and causes severe renal damage, i.e. acute tubular necrosis (13, 17, 18). All these cases with severe renal damage received high total doses of streptozotocin 15–36 g. The survival period in these reports varied between 7 days and 4 months. 3) The optimal effects of streptozotocin can be obtained by rather slow i.v. injection of the drug. The best remission results were obtained by a daily dose of 1–2 g and avoiding either a too rapid or too slow rate of injection (5, 7, 15, 16). The patients had satisfactory remission times of 12–15 months with intermediate injection rates. 4) A suitably long interval between the streptozotocin injections is 1–2 weeks. If the drug is given daily for several days, severe nephrotoxicity develops (15).

On the basis of these criteria we decided on the following treatment schedule. (The patient had a pancreatic carcinoma with several large liver metastases and several clinical symptoms.) We infused 2 g of streptozotocin on each of 4 occasions at 2 week intervals. The fifth dose was injected one month later. The two final doses were given after streptozotocin free periods of 3 months (Fig. 1).

The only side effects were vomiting, nausea and abdominal pains directly after the injections. We gave a very potent procainamide derivative (Primperan® methoclopramide chloride) 10 mg×3 i.v. daily and this new drug completely abolished all gastrointestinal side effects. During the whole period of treatment all liver enzyme, kidney and haematological laboratory data were completely normal. The original pancreatic tumour and the liver metastases disappeared and the patient seems to have been clinically cured during a 22 month period since the commencement of streptozotocin therapy. As far as we know this is the first patient in Sweden who has had a very long remission after carefully controlled streptozotocin treatment.

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The Postinfarction Clinic in Goteborg, Sweden

A Controlled Trial of a Therapeutic Organization

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ABSTRACT Since Jan 1, 1968 a Postmyocardial Infarction Clinic has been operating in Goteborg Sweden The methods used have been presented previously in this journal The present study compares 96 male postinfarction cases, 57–67 year-old, treated at the Postmyocardial Infarction Clinic and a random sample of 85 patients not treated at the clinic The mortality did not differ between the groups but there was a significant difference with regard to non fatal reinfarction The reasons for this are only partially explained by better control of accepted cardiovascular risk factors in the group treated at the clinic Cessation of smoking was vigorously recommended but lipid lowering, antiarrhythmic or anticoagulant drugs were never used in this group The results indicate that formalized management of homogeneous patient groups may achieve a general reduction of recurrences

The risk of sustaining a myocardial infarction has been shown to be increased in individuals who have previously suffered an infarction The risk of sudden death is equally high (8 15) Many of the survivors of a myocardial infarction are left with a considerable degree of invalidism (3 17 20) In the light of these circumstances a Postinfarction Clinic was established in Goteborg with the purpose of providing systematic follow up and treatment of infarction patients (11 12 18)

The aim of the present study was to investigate whether the rate of reinfarction or mortality in patients treated at the Postinfarction Clinic differed from that in patients managed in the traditional way for Goteborg

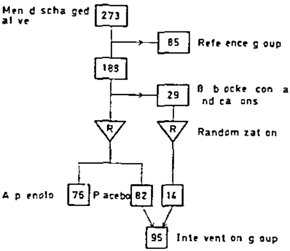
PATIENTS AND METHODS

The study comprised 273 men aged 57–67 years discharged from hospital alive after a myocardial infarction during 1970–71 Men born on dates ending in 3 6 and 9 were excluded in order to constitute a reference group These 85 patients were referred to other physicians for continued follow-up The remaining 188 patients were referred to the Postinfarction Clinic (5) They participated in a controlled study on the effect of chronic treatment with alprenolol (16 21) The patients were allocated randomly to treatment with alprenolol or a placebo The reference group was compared with the placebo group of 96 men The latter intervention group includes half the men ($n=14$ randomly selected) excluded from the β blocker study because of contraindictions (Fig 1

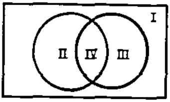
The patients were divided into four subgroups using criteria based upon findings during the period of acute treatment in hospital (Fig 2) (3 15) The presence of one criterion was sufficient for a patient to be referred to the relevant subgroup Table I shows the distribution of patients in the intervention and reference groups by subgroup The patients were comparable with respect to this prognostic classification The prevalence of previous infarction before the start of the study was the same in the two groups 25%

The patients in the intervention group were contacted during the period of acute care by one of the physicians who conducted the study The first control examination at the Postinfarction Clinic took place within one week after discharge The routine for subsequent control examinations and treatment was designed to ensure reproducibility At the control examinations by a physician 1 3 12 24 48 72 and 104 weeks after discharge from hospital a detailed history and the patient s physical status were recorded Intermediate control examinations were carried out by a specially trained nurse who collected case history data by means of a questionnaire measured the patient s BP and performed certain blood tests Thus control examinations at the Postinfarction Clinic took place at fixed intervals (every 6 weeks) or as indicated by the clinical situation The duration of follow up was two years In order to ensure uniform collection of data special computer records were used throughout Predetermined criteria were applied for intervention in the event of symptoms complications and presence of certain risk factors (5)

The study was performed by two physicians and one nurse who continuously trained in order



F g 1 Recruitment of patients to reference and interven tion groups



F g 2 Cntena for allocation of patients to subgroups One cnterion to be fulf lled for specif c subgroup referral *Subgroup I* No extens ve cardiac damage *Subgroup II* Mechanical damage to myocard um 1) relative heart volume ♂>450 ♀>400 ml/m² BSA 2) GPT>40 U during the first 3 days 3) BT>38°C during the first 3 days 4) trans ent atrial flutter and f bnllation *Subgroup III* Elec trical cardiac damage 1) VPB frequency>5/min 2) ven tricular tachycard a or ventricular f bnllation 3) AV blocks (*a*) PQ>0 24 sec (*b*) type II (*c*) type III *Subgroup IV* Combined electromechanical damage (subgroups II and III)

formity when filling in the form and reduce observer varia tion The same variables were investigated in a uniform manner at each f xed control examination The intake of tablets was controlled by nterv ew as well as by checking the number of remaining tablets

Deaths and non-fatal re nfarctions were recorded con tinuously by the Myocard al Infarction Register (4) The same criteria of diagnos s for non-fatal re nfarction were appl ed as for acute myocardial infarction at the beginning of the study Re nfarctions were regarded as non-fatal if the patient surv ved for more than 28 days All patients who d ed were examined post mortem and the cause of death was establ shed accord ng to WHO recommenda tions (International Class f cation of Diseases Geneva 1965)

The two phys cians who performed the study were never involved in the diagnos s of infarction or determina tion of the cause of death these data were obtained from e Myocardial Infarction Register (4) Only one end nt either non-fatal re nfarction or death was poss ble r each patient

Readmiss ons for suspected re nfarction card ac de compensation arrhythmias or other complications of in farction were recorded in the intervention group at control examinations at the Postmyocardial Infarction Cl nic The corresponding data for the reference group were recorded at the end of the study after examination of the case records and interviewing the patients

The signif cance of differences between the groups was tested using the χ^2 test.

RESULTS

All but one of the patients kept appointments for the regular control examinations at the Postmyocardial Infarction Cl nic The exception survived the fol low-up period without reinfarction The patients in the intervention group showed good compliance with the prescribed treatment regimen The results of tablet-counting showed that 80% of the patients took at least 90% of the prescribed doses (16)

Statistically significant reductions of non-fatal reinfarct ons and new coronary events (coronary deaths plus non fatal reinfarct ons) were demon strated during the two-year follow up (Table II) Readmission to hospital for one of the reasons above was equally frequent in both groups There were 90 readmissions in the intervent on group and 82 in the reference group The mean duration of treatment for these reasons was 1 0 day per ind vidual per year in the intervention group and 0 9 days in the reference group

COMMENTS

During the 1960s the hospital mortal ty from myocard al infarct on fell It was previously h gh 30–40% (9) The establishment of coronary care units (CCU) has in many centres led to a reduct on of the mortality at best by 50% (6 10) Less than half of all acute deaths occur in hosp tal Great effort has

Table I Distribut on of pat ents (%) accord ng to subgroups

Subgroup*	Intervention group	Reference group
I	25	25
II	49	48
III	3	1
IV	23	26

* According to F g 2

Table II *End points during the two-year follow up*

	Intervention group (N=96)		Reference group (N=85)		
	n	%	n	%	χ^2 test
Non-fatal reinfarction	11	11	22	26	p<0 025
Coronary deaths	9	9	13	15	n s
All deaths	12	13	14	16	n s
New coronary events	20	21	35	41	p<0 005

been devoted to improving hospital care constructing advanced monitoring equipment and developing potent drugs above all for the treatment of arrhythmias At present it would seem difficult to achieve major therapeutic advances as regards the acute hospital mortality This has led to efforts to reduce the mortality outside hospital

A retrospective study of men in Goteborg aged below 50 years who survived a primary myocardial infaction during 1948–65 showed that their medical management with respect to known risk factors such as high BP smoking and hypercholesterolaemia was very heterogeneous One third of the patients were not even under the control of a physician (7) In the light of these findings it was natural to start an outpatient clinic for uniform follow up of postinfarction patients—the Postmyocardial Infarction Clinic (12 18)

The present study is based on a total material from a defined group of infarction patients in Goteborg It has been shown that there is no pronounced age or sex-dependence with respect to reinfarction or deaths during the first two years after a myocardial infarction (14) It is therefore probable that the reduction in the rate of reinfarction observed in this study may be generalized to apply also to women and individuals aged below 57 years In the present study the patients in the intervention group had close contact with the staff of the Postmyocardial Infarction Clinic At control examinations they were repeatedly instructed about the measures they should take in the event of various symptoms occurring It is therefore possible that the patients may have come under treatment at an early stage while various conditions were susceptible to treatment and reversible This direct contact was maintained by frequent control examinations largely carried out by the specially trained nurse who had a high degree of independence (5 16) Despite this close contact between patients and the clinic re admissions were not more frequent in th n tion group

The reasons for the better results ... tion in reinfarction in the intervention group are not clear The main difference between the two groups was in the incidence of non fatal reinfarction This risk is not related t the sever y of the clinical course (13) Antiarrhythmic age ts lip d lowering drugs and anticoagulants were n t use in the intervention group (16) Vigorous antism k measures were taken however and 3 month infarction 60% of the smokers in the interv n group had stopped smoking (19) Stopping smoking has been found to prevent reinfarction bu this does not explain the results in the present study In this age group only about 50% of the patients were smokers at the time of infarction Some of the patients in the reference group probably also stopped smoking as a result of routine advice during the period of acute treatment in hospital though to a lesser extent than the patients in the intervention group (6)

The mortality during the first two years after a myocardial infarction is strongly correlated to the severity of the clinical course (13) The finding that the difference in the incidence of coronary death between the groups is smaller than the difference in the rate of non fatal reinfarction was therefore not unexpected Stopping smoking has been shown however also to reduce the mortality in patients who were smokers at the time of onset of infarction (19) For the reasons above this can play only a minor role in the present study

Only chronic treatment with β-blockers has so far been shown to reduce the long term mortality in patients after complicated myocardial infarction (1 2 16 21) No patient in the intervention group was treated with β-blockers

Irrespective of which factor exerted the greatest influence in the present study this investigation

shows that it is possible to influence the course after a myocardial infarction in an urban area by means of systematic follow up and treatment The situation is analogous to that immediately after onset of myocardial infarction Treatment in a CCU reduces hospital mortality by about half (6) A feature common to all studies on the effects of treatment in CCU is that it has not been shown which single factor is most effective In both cases it must be accepted that the total effect of a therapeutic organization is favourable

The method used in the present study cannot be transferred indiscriminantly to other hospital areas in Sweden or other parts of the world It should be easier however to design programmes for uniform management of certain categories of patients with in the existing systems of health care in other centres The establishment of rules for intervention with respect to complications symptoms and risk factors and continuous training of staff and patients are possible ways of achieving results

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Prognosis of Patients with Complete Heart Block or Arrhythmic Syncope Who Were not Treated with Artificial Pacemakers

A Long term Follow-up Study of 101 Patients

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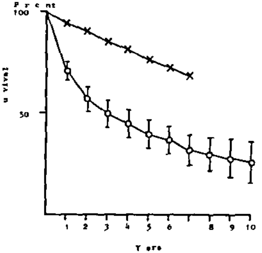
STRACT This paper reports the results of a :ospective study carried out with special reference the survival rate in a series of 101 selected cases luding patients with complete heart block (CHB) abined or not combined with Adams-Stokes at ks and patients with arrhythmic syncope with ECG evidence of CHB All these patients were ated in our Department during 1958–68, none ng artificially paced Twenty seven patients were re at the end of the follow up i e 6–15 years er admission to this Department on account of syn al episodes or CHB The survival rate—higher in nales than males—was lower in the cases of CHB nbined with Adams Stokes attacks than in the es of asymptomatic CHB This applied also to the tances in which a complicating disease such as haemic heart disease (IHD) hypertension, dia es digitalis intoxication or cardiac enlargement existed The survival rate in the 68 cases of CHB s higher at one year (68%) as well as at 5 years %) than that reported by other investigators hen assessing the survival rate in cases treated th artificial pacemakers, it is important to study individual case histories with special reference a previous or coexisting condition such as IHD, pertension, diabetes or the presence of cardiac en gement The present results support the view that indications for treatment with artificial pacing ould be wide, albeit that the prognosis in this series s more favourable than might have been an ipated from observations by others

rmanent artificial pacing has radically changed e prognosis of patients with complete heart block HB) combined or not combined with arrhythmic ncope (2 9 11, 13 21) The incidence of cases CHB combined with arrhythmic syncope treated with artificial pacemakers varies from country to country (12) probably due to differences in the re sources required Moreover opinion is divided as to the therapeutic value or artificial pacing When this treatment was introduced at the end of the 1950s it was used exclusively at least in Sweden in cases of CHB combined with arrhythmic syn copes (14) Subsequently the indications have been widened and at present symptomatic bradycardia appears to be generally accepted as an indication for artificial pacing irrespective of the underlying disturbance of rhythm Treatment by artificial pace makers is being used increasingly in cases of asymptomatic CHB because these patients carry an adverse prognosis (11) Paroxysmal ventricular tachyarrhythmia has likewise been successfully treated with artificial pacing with a high stimulation rate so-called overdriving (21)

Pacemaker therapy has been used at the Depart ment of Thoracic Surgery Karolinska Hospital since 1958 (8) The present paper reports the prog nosis of the patients with CHB combined or not combined with Adams Stokes attacks or with ar rhythmic syncope in the absence of ECG evidence of CHB who were not artificially paced All these patients were admitted to our Department in 1958–68 During that period 432 patients were referred from this Department to the Department of Thoracic Surgery for long term artificial pacing

MATERIAL AND METHODS

From 1958 to 1968 116 patients with CHB combined with Adams Stokes attacks or with arrhythmic syncope with



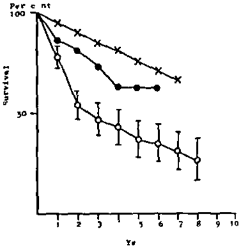


Fig 1 Left Cumulative survival rate (±2 S D) in the total material (O—O) compared with an age and sex matched control group (×—×)

Right Survival rate in the 68 patients with complete heart block (O—O) compared with 260 patients paced in Stockholm in 1962–68 (●—●) and with a control group of the same age and sex (×—×)

out any ECG evidence of CHB were treated in this Department None of these patients was artificially paced Their hospital records were reviewed with special reference to type of arrhythmia presence or absence of Adams Stokes attacks or VEBs cause of arrhythmia and coexistence of a myocardial lesion or any serious extracardial disease The part played by these factors in prognosis was then investigated The local parish offices which register births deaths marriages and other personal data were requested to report whether the patient concerned was alive or dead In 1974 information about these points was obtained in all cases The hospital records of 15 patients 5 of whom were alive at the end of the follow up were either not available or incomplete and were therefore excluded

Of the 101 patients presented in this paper 59 were and 42 women Their ages ranged from 18 to 93 years (mean of the men 67 (±14 S D) of the women 64 (±15 S D)) at the time of hospitalization

RESULTS

Survival

Twenty seven patients 11 men (18%) and 16 women (38%) were alive and 74 were dead at the end of the follow up ($p<0\ 05$) Eight patients had died less than two weeks after CHB had been recorded for the first time or after the first Adams Stokes attack had occured The follow up time calculated from the first occasion on which the conduction defect was documented electrocardiographically or Adams Stokes attacks occurred to the date when the answers from the parish registers were received was 6–16 years

Cumulative survival

The cumulative survival was determined in the whole case material as well as in the patients with CHB The difference between these two groups with respect to survival is strikingly small (Fig 1) Survival was also compared with that in a population group of the same age and with that in patients who were treated with long term artificial pacing in this Department in 1962–67 (7)

Type of arrhythmia

CHB was demonstrated electrocardiographically in 68 patients 33 had syncope combined with one or several of the following conditions AV block sinus bradycardia (<40/min) nodal bradycardia numerous VEBs (>5/min) ventricular tachycardia ventricular fibrillation and/or pulselessness on one or several occasions while treated in this Department (Table I)

The mean age of the patients with electrocardiographically demonstrated CHB was 67 years (±15 S D) and of the patients without CHB 66 years (±13 S D) while they were in hospital In 34 of the 68 patients with CHB this conduction defect was constant and a further 34 patients occasionally had AV conducted heart rhythm after CHB had been demonstrated electrocardiographically Eight (24%) of the patients in the former group and 9 (25%) in the latter were alive at the end of the follow up Of the 35 patients with CHB not demonstrated by ECG 10 (30%) were alive at the end of

le I *Type of arrhythmia and arrhythmic syncope*

	No of cases	Alive n	Alive %	Mean age ±S D (y)
CHB and syncope	41	7	17	68±12
CHB no syncope	27	10	37	65±17
2nd degree AV block and syncope	9	4	44	64±13
VF VT or VEB and syncope	12	5	42	62±11
Sinus arrest and syncope	6	0		80±10
Nodal bradycardia and syncope	2	0		
Pulselessness and syncope	4	1		60±16
Total	101	27	27	67±14

the follow up The 3 patients with nodal bradycardia and 2 of the 4 patients who had had episodes of pulselessness while in hospital were dead at the end of the follow-up The most favourable prognosis was found among the patients with second degree AV block or sinus arrest combined with syncopal episodes In 11 patients with syncope the underlying disease was thought to be ventricular tachyarrhythmia Four of the latter patients showed ECG evidence of ventricular tachycardia (5 or more consecutive complexes of at least 0 12 sec their configurations differing from the other ventricular complexes) and a further patient had ventricular fibrillation Six other patients had numerous VEBs (>5/min) or multifocal ectopic beats and syncopal episodes but were not examined by ECG during the latter Six of these 11 patients were alive but only one of the 5 patients with electrocardiographically confirmed ventricular tachyarrhythmia was alive at the end of the follow-up

This series included 4 patients who were pulseless on one or several occasions while in hospital or in whom there was auscultatory evidence of cardiac arrest One of them suddenly lost consciousness on three occasions while in hospital On one of these occasions the patient was pulseless and no heart sounds were heard This patient was alive at the end of the follow up Another patient had more than 20 syncopal episodes before admission On one occasion he was pulseless for about 10 sec while in hospital In a further patient who was admitted on account of syncope there was auscultatory evidence of cardiac arrest during at least 5 sec This patient had experienced some 10 syncopal episodes before admission A fourth patient treated in the hospital because of syncopes had also repeated episodes of pulselessness of about 5 sec duration The 3 last mentioned patients were dead at the end of the follow up

Acquired CHB with and without Adams Stokes attacks

Forty six per cent of the patients with CHB uncombined with Adams Stokes attacks survived compared with 26% of those in whom such attacks accompanied CHB (Table I) If the 5 patients with presumably congenital CHB two of whom had Adams Stokes attacks are excluded 40% of the patients with CHB uncombined with Adams Stokes attacks survived compared with 24% of those with CHB plus Adams Stokes attacks The difference is statistically significant ($p<0\ 01$)

Correlation between prognosis aetiology and past or coexisting serious cardiovascular disease

The dividing line between the aetiology of the disturbance of the conduction system and a previous or present complicating cardiovascular disease is not rigid and it is virtually impossible to make it so Coexisting cardiovascular diseases such as angina pectoris and/or hypertension need not necessarily be the cause of the conduction defect

The cases of second degree AV block and CHB in this series were divided into seven groups according to the presence of a coexisting cardiovascular disease and the presumptive cause of the disturbance of the conducting system (Table II) Patients with angina pectoris hypertension or diabetes who developed myocardial infarction were included in the myocardial infarction group None of the 5 patients with digitalis intoxication presented in Table II had a history of myocardial

Table II *Relationship between prognosis in cases of 2nd degree AV block or CHB presumptive cause of conduction defect and coexisting complicating disease*

	No of cases	Alive		Mean age ±S D (y)
		n	%	
Congenital CHB	4	4	100	34±17
Previous myocardial infarction	13	1	8	72±10
Angina pectoris hypertension diabetes	19	2	11	74± 6
Collagenosis lues myocarditis	10	4	40	63±10
Cause unknown absence of complicating disease	24	9	38	66±12
Digitalis intoxication absence of complicating disease	5	0		79±10
Rheumatic valvular disease	2	1		
Total	77	21		67±14

infarction angina pectoris hypertension or diabetes A further 2 patients developed digitalis intoxication in conjunction with IHD Both were dead at the end of the follow up Two patients developed CHB in conjunction with DC conversion In one of these the conduction defect was intermittent but he died from acute myocardial infarction 17 months later The prognosis of the patients with a history of myocardial infarction or digitalis intoxication was very unfavourable (Table II) Five patients who developed myocardial infarction within two months after CHB had been recorded electrocardiographically for the first time died The combination of a previous myocardial infarction and angina pectoris digitalis intoxication hypertension or diabetes mellitus was common Of the 37 patients with second degree AV block or CHB associated with one or several of these conditions 34 (92%) were dead at the end of the follow up compared with 36 (61%) patients whose past history did not include any of these conditions ($p<0.01$) The 4 patients with congenital CHB were not taken into account when assessing the prognosis in the latter two groups However the mean age of the patients in the former group was about 10 years higher than in the latter

Of the 4 patients with congenital CHB 2 had syncopal episodes which were thought to be induced by arrhythmia Four of the six deceased patients with collagenosis, lues myocarditis or valvular heart disease respectively, died suddenly One of these 4 patients had had myocarditis associated with severe arteriosclerosis of the coronary arteries and valves long standing pericarditis and the myocardium showed severe fibrosis One of them had myocarditis and died suddenly in her home at the age of 46 years Three months before her death she attended this Department to consider the question of pacemaker treatment At that time her heart size was 820 ml/m² BSA She was considered to be suitable for artificial pacing but was discharged because it was thought expedient to postpone treatment until the electronic components of the generator in use at the time had been improved

Serious extra cardial diseases

Five patients had cancer or a malignant blood disease but not chronic lymphatic leukaemia 3 of them showed in addition signs and symptoms suggestive of IHD All these patients were dead at the end of the follow up Nine patients had cerebrovascular lesions and 3 of them had IHD all were dead at the end of the follow up One patient with CHB had Adams Stokes attacks on about 20 occasions shortly before his death in hospital from symptoms suggestive of a cerebrovascular lesion Necropsy disclosed large subdural haematomas on both sides

The relationship between heart size and survival

Information about the heart size was available in 51 of the male patients Eighteen of them were alive at the end of the follow up their relative mean heart size being 495±167 ml/m² BSA in 33 patients who died the corresponding figure was 600±190 ml/m² BSA ($p<0.05$) Nineteen women were alive

at the end of the follow up the mean heart size being 445±170 ml/m² BSA in 18 women who had died it was 526±190 ml/m² BSA The difference is not statistically significant

DISCUSSION

Many investigations of the prognosis of patients with CHB with or without Adams Stokes attacks have been carried out (1 2 3 9 11 17 18 22 23 24) The survival rate one year after the diagnosis of CHB has been reported to be 50–60% Data on the survival rate in patients who were followed up for more than one year are scarce The patients presented in this paper were selected from a large group in which the other patients were artificially paced They are therefore neither comparable with those patients nor suitable as controls in subsequent series of patients identically treated

The patients in this series are all considered to be potential candidates for artificial pacing on the present-day indications for this treatment possibly with the exception of 4 patients with congenital CHB However 2 of the latter had had syncopal episodes and were therefore also potential candidates Nevertheless it was considered to be of interest to investigate whether the patients in this series who were potential candidates for treatment with artificial pacemakers showed characteristic features which distinguished them from those who were *not thus treated and were dead at the end of* the follow up All the patients who were alive at the end of the follow up were requested to report at this Department to take up artificial pacing for consideration

The hospital records of the patients in this series did not invariably afford information about the reasons why they were not artificially paced It was therefore not possible to study this point in more detail However it emerged from the records that some patients refused to be artificially paced In some cases arrhythmia was associated with a complicating disease such as cancer or a malignant blood disease which were no doubt considered to be contraindications In some cases the attending physician or the patient urged reasons for postponing the treatment and in some cases the attending physician in agreement with the patient considered the patient s advanced age to be a contraindication CHB without Adams Stokes attacks was not generally accepted as an indication for artificial pacing in Sweden at the time the patients in this series were treated in this Department (7)

At present a malignant blood disease with arrhythmic syncope is not considered to be a contraindication for artificial pacing Not only humanitarian but also practical reasons related to the care of patients with Adam Stokes attacks have contributed to modifying our view of the indications for this treatment It is well known that there is always a danger of the patient incurring fracture of the skull or being otherwise severely hurt in conjunction with an Adams Stokes attack Mental confusion of a patient with CHB and a slow heart rate does not necessarily contraindicate treatment with an artificial pacemaker on the contrary this symptom should strongly motivate this treatment (7) Cerebrovascular accidents may however occur in conjunction with Adams Stokes attacks One patient in this series thus had a subdural haematoma on both sides This condition should be considered in a patient who continues to have symptoms suggestive of a cerebral lesion after artificial pacing has been instituted

The mean age of the patients in this series 70 years was rather high The females were somewhat younger than the males but the difference in age was not statistically significant The survival rate was higher in the women than in the men There are two possible explanations of this difference firstly the difference between men and women in *the population in this respect and secondly the* difference between the sexes with respect to mortality from IHD According to the National Central Bureau of Statistics in Stockholm (1968) the remaining life expectancy in 1962 was 14 0 and 10 2 years for women aged 68 and for men aged 71 respectively Johansson (11) reported that the survival rate in a series of 204 patients with CHB who were not treated with artificial pacing was about 50% at one year The corresponding percentage in patients who were artificially paced was 90–95% at the end of a one year follow up (2 13 19 20)

The patients with CHB in our series did not differ from those with arrhythmic syncopes not showing this conduction defect with respect to survival (Fig 1) In view of the small number of patients without CHB it was not considered relevant to study these patients in greater detail here A comparison of the prognosis of the CHB patients with and without Adams Stokes attacks respectively

showed that the prognosis was in some cases more favourable in the former group (11) This was also observed in the present investigation

It has been shown that the prognosis of patients with CHB varies, depending on the underlying disease (3) According to Johansson (11) the prognosis of patients with CHB due to rheumatic heart disease , miscellaneous conditions or with an obscure cause of the conduction defect is much more favourable than of patients with CHB complicated by a coexisting disease of the coronary arteries or digitalis intoxication In the series reported by Johansson survival was strikingly high in the cases in which the cause of CHB was determined This observation prompted us to compare the survival rates of patients with CHB associated with IHD hypertension or diabetes and of patients with CHB not combined with any of these conditions It was found that the prognosis of the patients with CHB combined with one or several of these complicating diseases was signficantly poorer than that of the patients with CHB alone Admittedly these two groups in our series differed with respect to mean age However it is questionable whether the difference in age was the only factor involved in the difference in prognosis Johansson (11) found the survival to be low at 1 year in patients with CHB associated with one or several of the above diseases In this investigation the prognosis of such patients was still found to be poor at the end of a prolonged follow up

The combination of CHB and a disease of the coronary arteries does not necessarily imply that the latter condition is involved in the causation of block (6 15 16) It is possible that one of the 5 patients in our series who developed myocardial infarction within two months of the first occasion on which there was ECG evidence of CHB had previously a painless myocardial infarction which caused the conduction defect The long term prognosis of patients who develop CHB in conjunction with acute myocardial infarction is poor This applies also to patients with intermittent block (10)

Mortality in the cases of CHB in this series was higher in the first year of the follow up compared with the subsequent years This may have been due to the fact that the cause of arrhythmia was a coexisting undetermined disease which may have contributed to impair the prognosis The significance of coexisting diseases such as renal failure diabetes, cardiovascular lesions or heart failure for excess mortality has been demonstrated recently (3, 11) IHD coexisting with CHB is prognostically an unfavourable factor also in cases in which the patients are artificially paced (2 7) It is possible that the use of ventricular programmed pulse generators (QRS synchronized or inhibited) will reduce the excess mortality in patients with CHB combined with IHD because this type of pacemaker does not involve the risk of stimulation during the vulnerable zone of the cardiac cycle On the other hand it has been demonstrated that only artificial stimulation by fast rates so called overdriving prevents tachyarrhythmia (21) The development of a pacemaker capable of suppressing tachyarrhythmia is in progress Such pacemakers will make it possible to treat patients with ventricular tachyarrhythmia which is refractory to medical treatment

It has been reported that some patients with acquired CHB may live many years after the lesion has been diagnosed (2 p 75) However the prognosis of the patients with CHB in this series who were not artificially paced was comparatively poor at one year This is in keeping with the observations of other investigators

In earlier investigations with shorter follow up times than in ours it was found that in cases of AV block associated with IHD diabetes or hypertension the prognosis is poor This finding was confirmed here and it seems that cardiac enlargement too is an unfavourable prognostic factor

Survival at one year in the cases of CHB in our series was higher (78%) than that (50%) reported by Johansson (11) The mean age of the patients in his series was 68 years i e somewhat lower than in our patients Johansson reported however that there was no noteworthy difference between age groups in his series except those over 80 years

Although the survival rate in our series was higher at one year than reported by others it differed from that in the patients who were treated with artificial pacemakers (7) This was also true after a follow up of 5 years This observation is confirmed by Cosby and Bilitch (2)

In patients with CHB combined with arrhythmic syncope as well as in patients with AV block combined with heart failure as a result of slow heart rate treatment with artificial pacemakers is indicated to relieve the patients from incapacitating symptoms and to prolong their lives

ACKNOWLEDGEMENTS

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Announcements

Second International Postgraduate Course on Myocardial Infarction and Angina Pectoris will be held in Davos Switzerland March 20–26 1977

Organized by Dr B Pitt Associate Professor of Medicine The Johns Hopkins University School of Medicine Baltimore and Dr P Lichtlen Professor of Medicine and Cardiology Hannover Medical University West Germany in cooperation with lecturers from the USA and Europe

Sponsored by the American Heart Association

Further information (European participants) Prof Dr P Lichtlen Abteilung für Kardiologie Department fur Innere Medizin Medizinische Hochschule Hannover D 3000 Hannover BRD (Participants from overseas) Dr B Pitt Associate Professor of Medicine Division of Cardiology The Johns Hopkins Hospital Baltimore Maryland USA

The 18th Postgraduate Institute for Pathologists in Clinical Cytopathology is to be given at The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital Baltimore Maryland USA April 11–22 1977 The full two-week program is designed for pathologists who are certified (or qualified) by the American Board o Pathology (PA) or their international equivalents Th entire course is given in English Topics will be covere in lectures explored in small informal conferences an discussed over the microscope with the Faculty Appli cation is to be made before Feb 28 1977

Information J K Frost M D 610 Pathology Build ing The Johns Hopkins Hospital Baltimore Marylan 21205 USA

11th World Congress of Neurology will be held at the In ternational Congress Centre RAI in Amsterdam Th Netherlands, Sept 11–16 1977 on the following sub jects neuroimmunology neuromuscular disorders di turbances of consciousness and cognition geograph factors in neurology Languages used English French German Spanish

Organizing body and/or sponsor World Federation c Neurology

Deadline for abstracts March 31 1977, for complet texts Sept 10 1977

Secretariat c/o Holland Organizing Centre 16 Lang Voorhout The Hague The Netherlands

AM Prize 1976 awarded for the Implantation of the Cardiac Pacemaker

On the occasion of their 150th anniversary in 1975 the ...hen and Munich Insurance Company in Aachen ... the Aachen and Munich Prize for Technology ... Applied Natural Sciences to be awarded every year with 60 000 DM by an independent board of curators consisting of six professors of the Technical University of Aachen and the Technical University of Munich

This year the prize has been awarded to Rune Elmquist and Åke Senning

They were the initiators of the implantation of electric cardiac pacemakers and the prize is given in recognition of the great humanitarian importance of their fundamental progress in heart therapy The technical development was provided by Dr Rune Elmquist Swedish doctor and technologist Professor Dr Åke Senning surgeon and at present director of the Surgical Clinic of the University c Zurich was the first to implant the pacemaker in 195 Both winners have developed one of the most productiv therapeutical methods which represents one of th greatest achievements in medical progress over recen years

As a result hundreds of thousands of patients all ov the world now owe their lives to the pacemaker It n only increases their life expectancy but also improv their quality of life to a level that could not have bee achieved by treatment with drugs The patient notices th pacemaker so little that at times he completely forge his life depends on the well functioning of this technic device

The pacemaker therapy is a classical example of th benefits that arise from the close cooperation betwee engineering and medicine

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Systolic Time Intervals in Cardiac Tamponade

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ABSTRACT Systolic time intervals (STI) have been measured in three patients with cardiac tamponade The left ventricular ejection time (LVET), the pre ejection period (PEP) and the ratio PEP/LVET deviated significantly from the normal values All three parameters improved immediately after peri cardiocentesis and aspiration The total electro mechanical systole changed to only a minor degree Measurement of STI may be a valuable tool in the diagnosis and treatment of cardiac tamponade

The diagnosis of cardiac tamponade is usually based on clinical signs such as chest pain dyspnea distension of the neck veins tachycardia fall in systolic blood pressure low pulse wave and pulsus paradoxus The measurement of systolic time inter vals (STI) is a noninvasive method which gives results that correlate well to other hemodynamic parameters (11) Left ventricular ejection time (LVET) is shortened by reduced stroke volume and by increased myocardial contractility The pre ejection period (PEP) is a function of the left ventricular end diastolic pressure (LVEDP) and the aortic diastolic pressure (ADP) as well as myo cardial contractility and left ventricular filling (10) The ratio PEP/LVET has been found to correlate closely to the angiographically determined ejection fraction (EF) (2) We have measured total electro mechanical systole (QS2) and LVET and estimated PEP and PEP/LVET in three patients with cardiac tamponade before and after pericardiocentesis

METHODS

STI were obtained from simultaneous recordings of phonocardiogram (PCG) carotid pulse wave and ECG The PCG was obtained by a pick up (Elema Schonander) placed over the upper part of the sternum The carotid pulse was registered by a multipulse transducer (Elet tronica Trentina) QS2 was measured from the beginning of the QRS in the ECG to the beginning of the second heart sound in the PCG LVET was measured from the beginning of the upstroke to the dicrotic notch in the carotid pulse wave PEP was calculated as the difference between QS2 and LVET and the ratio PEP/LVET was estimated The paper speed was 100 mm/sec and each measurement was made to the nearest 0 5 mm (5 msec) The heart rate (HR) was estimated from the R R distance and QS2 LVET and HR were calculated as the mean of registrations in three systoles The values are presented as percentages of the normal values at different HR based on regressions described earlier (1) The regression equa tions were QS2=525–2 0 HR LVET=391–1 5 HR and PEP=133–0 5 HR The ratio PEP/LVET is not dependent on HR the mean value being 0 35 (S D ±0 05) The re gistrations were made with the patients in their beds and with the body elevated 20 Recordings were made imme diately before and after pericardiocentesis In one pa tient (no 1) with chronic pericardial effusion STI was registered during the course of hospitalization on days 3 6 10 15 and 21 On day 6 STI was recorded before and after pericardiocentesis It was difficult to obtain good recordings because of the patients anxiety and use of auxiliary respiratory muscles

CASE REPORTS

Case I

A 48 year-old man was admitted to the hospital with chest pains Serial ECG tracings showed signs of acute myo- cardial infarction and anticoagulant therapy (warfarin) was instituted

After 5 days the patient developed clinical signs of pericardial effusion The findings were confirmed by X ray and blood pool scanning Anticoagulant therapy was discontinued and vitamin K given After 2 days cardiac tamponade developed and pericardiocentesis was performed 240 cm³ blood was aspirated and the clinical condition improved immediately After one
revealed increase in the heart size Th

Table I *Systolic time intervals in patient 1 with pericardial effusion*

On day 6 he had symptoms of cardiac tamponade and pericardiocentesis was performed The normal ranges fo QS2 LVET and PEP are the calculated values at different HR=100%±2 S D PEP/LVET is x±2 S D

	Normal range	Day 3	Day 6[a]	Day 10	Day 15	Day 21
QS2	92–108%	107	96	94	100	112
LVET	90–110%	101	70	89	91	104
PEP	*85–115%*	*128*	*172*	*108*	*128*	*133*
PEP/LVET	0 25–0 45	0 430	0 850	0 420	0 485	0 435

[a] Registrations immediately before pericardiocentesis Registrations immediately after pericardiocentesis are given in Table II

clinical signs of tamponade but when pericardiocentesis was performed 600 cm³ blood was aspirated No registrations of STI were made on this occasion The *patient s general condition improved gradually until he* died suddenly four weeks after admission Post mortem examination revealed blood in the pericardial sac and the cause of death was thought to have been cardiac tamponade due to an acute bleeding Histological examination showed an angiomatous neoplasm in the epicardium (3)

Case 2

During the terminal stage of a squamous cell carcinoma of the right lung a 60 year old man developed clinical signs of heart tamponade Blood stained fluid 160 cm³ was withdrawn from the pericardial sac A few days thereafter the patient died and post mortem examination showed tumor infiltration in the pericardium

Case 3

*A 54 year old woman was admitted because of progres*sive dyspnea A chest X ray showed pleural effusion and her condition improved after aspiration Two weeks later clinical signs of heart tamponade developed and 200 cm³ blood stained fluid was aspirated from the pericardial sac Her condition improved immediately Subsequent cytological examination of the aspirated fluid revealed malignant cells and an adenocarcinoma of the right mamma was diagnosed later

RESULTS

STI in patient 1 with pericardial effusion are shown in Table I

PEP was slightly prolonged on days 3 15 and 21 and extremely prolonged on day 6 when the patient had clinical signs of cardiac tamponade At the same time LVET was shortened and PEP/LVET extremely high QS2 was within normal limits in all registrations STI immediately before and after pericardiocentesis are shown in Table II Before *aspiration LVET was extremely shortened PEP* lengthened and PEP/LVET high Immediately after aspiration all three parameters changed towards normal values The changes in QS2 were minor

DISCUSSION

STI in patients with cardiac tamponade reflect hemodynamic changes characterized earlier by other parameters (4, 5 7 8 9) The small stroke volume is responsible for the shortening of LVET and lengthening of PEP (4 5 8)

Reduction in myocardial contractility will tend to lengthen both QS2 LVET and PEP In cardiac tamponade dp/dt is reduced (7) In our patients only PEP is lengthened The cause of this may be the reduction in stroke volume which is the dominant factor and leads to shortening of LVET and to a minor degree *of QS2 The extreme lengthening of* PEP is a result of the reduction in both left ventricular filling and contractility Rise in LVEDP and

Table II *Systolic time intervals immediately before (B) and after (A) pericardiocentesis*

	QS2		LVET		PEP		PEP/LVET	
Pat no	B	A	B	A	B	A	B	A
1	96	97	70	90	172	117	0 850	0 445
2	93	84	*69*	*78*	*148*	*101*	*0 690*	0 450
3	87	93	71	85	132	120	0 650	0 480
Normal values	92–108%		90–110%		85–115%		0 25–0 45	

all in ADP in cardiac tamponade (7) will otherwise tend to shorten PEP but this is opposed by the reduction in left ventricular filling and contractility The high PEP/LVET indicates a low left ventricular F The aspiration of rather small volumes of fluid from the pericardial sac improves the ratio We see this as a confirmation of the finding that when the tension of the parietal leaf is lessened the cardiac function is immediately improved (6 8 9) Even if TI may be difficult to measure in patients with cardiac tamponade we have found the method to be of great value in the diagnosis and treatment of patients with this condition

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Mortality Pattern among Initial Survivors of Acute Myocardial Infarction Using a Life-table Technique

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BSTRACT The 5 year pattern of mortality among 75 immediate survivors of acute myocardial infarction (AMI) (mean age 65 years on entry) is described y a life table technique The risk of death was ghest during the early part of the follow up fter 3–4 years the prognostic influence of the AMI emed to be overshadowed by the age effect Special tention was paid to the incidence of sudden death fictive elimination of which was shown to reduce e risk of death by 9–22% during the different ars of the investigation period The absolute umber of sudden deaths was highest during the rly part of the follow up period but the relative nportance of this mode of death was approximately e same during the entire 5 year period after the MI

ollow up studies of initial survivors of acute myocardial infarction (AMI) have shown that 75–94% the patients subsequently die from a manifestaon of them ischaemic heart disease (IHD) (2 3 6) mong the IHD deaths it is sudden death variously efined which has attracted most interest as it has een assumed to be caused by ventricular arrhythmias which might be avoided by antiarrhythmic ophylaxis

The aim of the present study has been to describe e pattern of mortality in a group of AMI survivors y means of a life table technique The different uses of death were registered and competing sks were calculated in order to assess to what ttent the prognosis might improve if effective ophylaxis could be provided against sudden lethal rhythmias

PATIENTS AND METHODS

From 1968 to 1970 475 patients were discharged alive after a first AMI or a reinfarction treated initially at the CCU Serafimerlasarettet Stockholm The group has been described in detail elsewhere (5) There were 312 (66%) males and 163 (34%) females The mean age was 65 years on admission the distributions by age and sex are shown in Fig 1

A follow-up was made on Dec 31 1973 to study the mortality rates during the first 3–6 years after admission As only a few patients had been followed for 6 years the results presented below are confined to the first 5 years An attempt was also made to differentiate between primary causes of death paying special attention to the IHD group

The following classification was used 1) IHD (*a*) Sudden death defined as death within 2 hours of the onset of final episode (*b*) Death within 2–24 hours (*c*) Death >24 hours after the onset of the final episode 2) Not IHD 3) Unknown (*a*) Deaths from unknown cause (*b*) Lost cases The classification was based upon data from death certificates and hospital records For patients who had died at home or elsewhere except in a hospital the routine police records were checked for relevant information

The life table methods used have been described by Chiang (1) and are based on a stochastic approach Chiang has developed formulas for the study of competing risks For this purpose the following basic quantities were used

Crude probability (Q_{xd})

The probability of dying from a specific cause (*d*) in the presence of all other risks i e the risk of dying or being lost multiplied by the fraction of deaths due to the specific cause

Partial crude probability ($Q_{xd \cdot r}$)

The probability of death from a specific cause when another risk (*r*) is eliminated If all other risks are eliminated the estimated risk is equal to the net probability

Table I *Distribution of patients according to survival and withdrawal status and to mode of death*

Interval after admission	No of pats at the beginning of the interval	No of pats not due for withdrawal in the interval							
					Modes of death				
		Total	Survivors	Deaths	<2 h	2–24 h	>24 h	Not IHD	Unknown and lost
1– 6 mo	475	475	422	53	13	10	15	7	8
7–12 mo	422	422	390	32	7	4	12	5	4
1– 2 y	390	390	348	42	4	8	12	16	2
2– 3 y	348	343	311	32	6	3	9	9	5
3– 4 y	311	197	179	18	4	1	7	5	1
4– 5 y	179	68	58	10	1	2	5	2	0

Net probability (q_{xd} $q_{x\cdot d}$)

The probability of death if the risk studied is the *only risk* acting or the probability of dying when the specific cause is *eliminated* These rates give estimates of corresponding risks in a hypothetical population where the cause studied is the only cause or does not exist at all

By comparing the net probability of dying or being lost ($q_{x\cdot d}$) when a specific cause (d) is eliminated with the probability of dying or being lost (q_x) one visualizes the relative importance of the specific cause The quantity

$$100x \frac{(q_x - q_{x\cdot d})}{q_x}\%$$

also estimates an upper limit for gain in survival if the population studied could be changed into a theoretical population in which the specific cause does not exist

RESULTS

Altogether 180 of the 475 patients died during the follow up and 9 were lost The distribution of cases according to withdrawal and survival status as well as to primary causes of death is presented in Table I Thirty five (19%) of the deaths were classified as sudden and the majority of these occurred during the first year after admission to hospital

Forty four patients (24%) had died from other causes than IHD Owing to lack of information the cause of death could not be verified for certain in 6 of the 180 deceased patients In another 7 patients the cause was IHD but the interval between the onset of the final attack and death could not be determined For practical reasons these patients were also classified under unknown in Table I

The net and the partial crude probabilities of dying from different causes in the follow up intervals are presented in Table II

Fig 2 shows the cumulative survival rates for the whole patient group After one year 82% of the patients were alive and after five years 56% For comparison Fig 2 also shows the expected age and sex-corrected survival rates calculated from the statistics from 1966–71 published by the Swedish National Central Bureau of Statistics

The mean age of the patients on admission to hospital was 65 years The variations in mean ages of the survivors and the deceased in each follow up year are shown in Table III The mean age of the

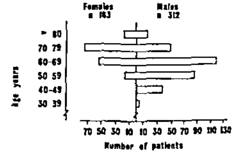


Fig 1 Age on admission to hospital and sex distribution of 475 immediate survivors of AMI

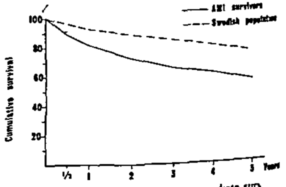


Fig 2 Cumulative survival among immediate survivors of AMI compared with the age population

No of pats due for withdrawal in the interval		
Total	Survivors	Deaths
5	4	1
114	114	
111	110	1

deceased increased from 69 to 76 years The latter age is close to the mean age at death in the general Swedish population The age distribution of the deceased within each follow up interval is further illustrated in Table IV where the younger age groups show a relatively high mortality rate during the early part of the follow up

The risks of death within different intervals of the follow up have also been compared with the expected values calculated from the Swedish population (Fig 3) During the first year after the AMI the risk of death was 17 9% The risk diminished during the next three years but rose slightly during the fifth year As expected the risk of death was higher in the patient group than in the population at large

The risks of death from different causes during the follow up years are presented in Fig 4 The risk of dying suddenly was 4 2% during the first year after the AMI and during the following years it stabilized around 1% As to dying not suddenly from IHD the risk was also highest during the first year after discharge it then diminished but rose again in the fifth year after the AMI as did the risk of death from other causes

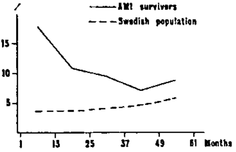


Fig 3 Risk of death among survivors of AMI compared with the expected values of the population

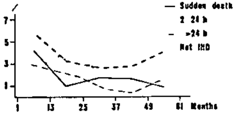


Fig 4 Risk of different modes of death among immediate survivors of AMI

The extent to which the probabilities of dying in the different intervals are reduced if sudden death is eliminated is shown in Table V The importance of sudden death compared with the other modes of death varies from 9 to 21%

DISCUSSION

In life table methods in common use lost cases and withdrawals are treated similarly However since all individuals in a follow up study entering a specific interval (x $x+1$) are exposed to the risk

Table II *Net and partial crude probabilities (per 100 000) of different modes of death among survivors of AMI*

Interval after admission	Partial crude probability					Net probability					Risk of dying per 100 000
	<2	2–24	>24	All IHD	Not IHD	<2	2–24	>24	All IHD	Not IHD	
1– 6 mo	2 743	2 110	3 165	863	1 477	2 860	2 208	3 293	8 746	1 550	10 561
7–12 mo	1 665	951	2 854	569	1 189	1 710	981	2 914	5 743	1 225	7 583
1– 2 y	1 025	2 051	3 076	3 448	4 102	1 079	2 147	3 203	6 968	4 248	10 769
2– 3 y	1 745	872	2 618	3 041	2 327	1 807	908	2 699	3 062	2 402	8 438
3– 4 y	1 577	394	2 761	3 615	1 972	1 620	408	2 818	3 628	2 021	6 706
4– 5 y	808	1 616	4 040	4 116	2 424	843	1 678	4 143	4 140	2 507	8 888

* Risk of being lost eliminated

Table III *Mean age of survivors and deceased in different intervals*

Interval (y)	Mean age at entry into interval		
	Survivors	Deceased	All
0–1	64 1	69 1	65 0
1–2	64 5	70 4	65 1
2–3	65 0	71 2	65 5
3–4	65 6	72 2	66 0
4–5	66 2	76 1	66 6

of dying or being lost but only some will be with drawn it is clear that lost cases and withdrawals do not belong to the same category (Fig 5) The rele vant basic probability and the one which should be computed is the *risk of dying or being lost* within a certain follow up interval Elimination of one cause of death does not imply that the probability of survival in a specific interval increases by the same amount This is due to an increased risk of dying from other causes The problem is to estimate the true benefit if a specific cause of death is elimi nated The method presented by Chiang (1) enables us to make the desired distinctions and to study competing risks which are of interest when investi gating late prognosis and the impact of recurrences of illness

The present 5 year survival rate of 56% among initial survivors of AMI is in accordance with findings in other studies (3 7) However precise comparisons are difficult to make because of dif ferences in the composition of patient groups As cipated the AMI survivors ran a higher risk of than expected from population figures cor rected for sex and age The risk of death was highest during the first year after admission to hospital and decreased during the next few years

Table IV *Age distribution (%) of the deceased within the different intervals*

Age group (y)	Interval (y)				
	0–1	1–2	2–3	3–4	4–5
≤49	3 7	2 4	–	–	–
50–59	12 3	7 1	13 7	11 8	9 1
60–69	37 0	33 3	17 2	17 6	18 2
70–79	29 6	42 9	44 8	35 3	36 4
≥80	17 3	14 3	24 1	35 3	36 4
No of deaths	85	42	32	18	10

Table V *Reduction of risk of death when sudd death is eliminated*

Interval after admission (y)	Risk of dying or being lost (per 100 000)		
	Total	When sudden death is eliminated	Reductı of risk (%)
0–1	17 895	13 996	21 6
1–2	10 769	9 796	9 1
2–3	9 544	7 879	17 4
3–4	7 087	5 557	21 6
4–5	8 888	8 114	8 7

When the mean ages of the deceased and th survivors were compared for the different perio of the present follow up the results indicated comparatively high number of deaths among th younger patients in the first years after AMI Th finding of a more rapidly increasing mean a among the deceased than among the survivors the follow up intervals indicates that the ri associated with being a survivor of AMI initial surpasses that of being of old age As the follow up becomes longer however chronological a gains in importance This tendency may expla the increase in the risk of death during the lat part of the present follow up period (Fig 3) In national follow up study of 642 survivors of AM in Denmark (3) a similar tendency was register

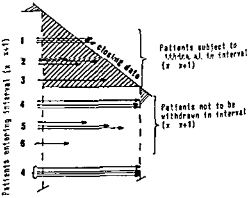


Fig 5 Differences betw follow up studies withdrawn in the be withdrawn die (2) and some Among i interval i e bef and enter the may be lost (6)

from the fourth follow up year and was interpreted as evidence of the increasing normal mortality in older age groups

Looking at the different modes of death (Fig 4) the incidence of all except sudden death and to some extent not IHD developed in the same way during the years of the investigation period The absolute risk of sudden death was highest during the first year after the AMI and then stabilized around 1% per year If sudden death could have been eliminated the probability of dying within each year would have diminished by up to 22% Although the small absolute numbers of sudden deaths especially during the second and fifth years in the present study, make this figure sensitive to random variations it does seem lower than those found in some other investigations (4 6) Varying definitions of sudden death as well as different age distributions of the patient groups make comparisons difficult But the clinical need for effective forms of safe prophylaxis against sudden serious ventricular arrhythmias in patients with known IHD is repeatedly stressed

The practical difficulties of classifying the cause and mode of death should not be overlooked In spite of the precautions taken when collecting the present material the unexplained increase of not IHD deaths during the second year of the follow up period (Fig 4) makes it necessary to consider the adequacy of the classification The choice of an interval of up to 2 hours between onset of acute symptoms and death for the definition of sudden death was a practical one Very early during the study it was evident that although all possible data were searched a limit below 2 hours could not be justified It is possible that the difficulty of determining the exact time of death is less poignant in strictly prospective studies (2)

The mortality pattern among initial AMI survivors needs further study An investigation of a larger patient group than the present one might help to clarify the following questions 1) What differences are there in the relative importance of sudden death in different age and sex groups of AMI survivors? 2) What might be the gain expressed in life expectation if the risk of sudden death could be diminished among survivors of AMI?

ACKNOWLEDGEMENT

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such a procedure not more than about 16% of the total fat cell number is removed He stated that the degree of filling of the adipocytes is of greater importance for other regulation of food intake or energy balance than is the number of cells

The results of our study suggest that the degree of filling of adipocytes did not influence the fractional removal rate of TG from blood under our experimental conditions

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The Intercorrelation of Serum Cholsterol, Cigarette Smoking and Body Weight

The Oslo Study

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ABSTRACT A screening for coronary risk factors in 18000 Oslo men yielded 16525 healthy men aged 20–49 The intercorrelation of serum cholesterol body weight and cigarette smoking was found to be more pronounced than described in other stud es Increasing daily exposure to cigarette smoke in the order never smoker ex smoker non inhaling smoker inhaling smoker and present non filter smoker was parallelled by increasing cholesterol levels but not by increasing body weight Daily cigarette smokers had lower body weight and higher serum cholesterol values than never cigarette smokers with the exception of the 20+ cigarette smokers who had higher serum cholesterol values and body weight than the never-cigarette smokers As regards ex cigarette smokers both body weight and serum cholesterol tended to increase with the number of cigarettes smoked before quitting Possible explanations of these f ndings are discussed

C garette smoking elevated serum cholesterol and excess body weight are well established risk factors for coronary heart d sease (CHD) Especially cholesterol and number of cigarettes smoked a day have proved to be powerful predictors of CHD in young and middle aged men although their causal mportance is st ll be ng d scussed (10 12 14 15 17 18)

When evaluat ng the pred ctive power of a certain risk factor it should be kept in m nd that a var a tion in the factor can be influenced by a simultaneous variation in other poss ble risk factors Thus t s important to test poss ble intercorrelat ons of risk factors A posit ve correlat on between serum cholesterol and body weight has been found in several studies (5 8 10 13 18) whereas until recently the relationsh p between the three major coronary risk factors—serum cholesterol blood pressure and cigarette smoking—has been regarded as unimportant or even non-existent (17 18)

In the present study the intercorrelation of cigarette smoking serum cholesterol and body we ght is elucidated

MATERIAL AND METHODS

In the Oslo Study all Oslo men aged 40–49 and a 7% random sample of men aged 20–39 were inv ted to a screening for coronary risk factors During the period May 1972 Dec 1973 about 18000 men were exam ned The screening procedure and the laboratory methods together with a detailed analys s of the results have been reported earl er (13) In this paper the term "main group comprises men without known card ovascular disease or d abetes and the study deals with these men The reason why we have analyzed this group separately s that when studying intercorrelations of risk factors t is of great importance to make the studied group as homogeneous as poss ble

Information about the smoking hab ts of the men was obtained by a self administered quest onnaire controlled and completed at the screening station

In the statist cal analys s the d fferences between means have been tested by a modif ed Student s *t* test accounting for unequal variances and numbers of groups

RESULTS

Table I presents the mean cholesterol values in groups of present-cigarette smokers ex-c garette

Table I *Mean serum cholesterol (mg/100 ml) by age in different groups of cigarette smokers and nc cigarette smokers*

Age group		Never cigarette smokers	Present-cigarette smokers Total	Inhalers	Non inhalers	Filter	Not filter	Ex cigarette smokers
20–24	n	132	191	186	4		141	46
	Chol	195 3	201 5	202 1	180 7		201 9	203 5
25–29	n	215	249	243	6	66	181	101
	Chol	217 5	230 0	230 5	211 2	229 0	230.3	270 6
30–34	n	109	167	158	9	46	121	97
	Chol	232 8	247 8	247 7	250 6	249 7	247 1	245 1
35–39	n	100	181	169	12	41	137	90
	Chol	232 5	261 1	261 9	251 2	262 9	261 4	243 9
40–44	n	1 504	3 114	2 941	169	796	2 297	2 081
	Chol	255 0	269 2	269 7	261 4	265 0	270 7	261 4
45–49	n	1 377	3 669	3 443	221	818	2 821	2 475
	Chol	265 1	274 4	274 6	271 1	269 7	275 8	267 7
Total	n	3 437	7 571	7 140	421	1 816	5 698	4 890
	Chol	253 1	268 1	268 3	264 5	263 6	269 5	262 6

smokers, and never cigarette smokers Within each group there is a considerable age trend The lowest cholesterol value (195 mg/100 ml) is found in the 20–24 year old never cigarette smokers the highest (276 mg/100 ml) in the present non filter cigarette-smokers aged 45–49 In addition to this age trend there is a higher cholesterol level in present cigarette smokers compared with ex cigarette smokers and never cigarette smokers with the possible exception of some small groups of present-ci arette smokers in the two youngest age group

In present cigarette smokers there is a trend to wards a higher cholesterol level in the inhale compared with the non inhalers and in the nor filter cigarette smokers compared with those smol ing filter cigarettes Sufficient numbers for statist cal evaluation are found only in age groups 40–4 and 45–49 For inhalers versus non inhalers th

Table II *Mean serum cholesterol (mg/100 ml) by age in different groups of daily cigarette smokers an in never cigarette smokers*

Age group		No of cigarettes consumed per day 1–4	5–9	10–14	15–19	20+	Unanswered	Total	Never cigarette smokers
20–24	n	13	49	78	37	13	1	191	132
	Chol	176 6	197 1	203 8	199 1	237 5		201 4	195 2
25–29	n	20	49	90	60	29	1	244	215
	Chol	203 7	212 7	234 9	244 1	231 5		229 0	217 4
30–34	n	11	24	52	45	34	1	167	109
	Chol	241 9	248 9	248 7	246 3	245 9		247 8	232 7
35–39	n	11	31	44	47	47	1	181	100
	Chol	256 2	246 1	273 3	258 8	261 1		261 1	232 5
40–44	n	204	523	985	677	719	5	3 113	1 504
	Chol	254 6	269 1	270 0	269 2	271 7		269 1	254 9
45–49	n	299	742	1 191	703	731	3	3 669	1 377
	Chol	262 0	270 2	277 8	274 5	277 8		274 3	265 1
Total	n	558	1 418	2 440	1 569	1 573	12	7 565	3 437
	Chol	254 7	264 4	270 0	268 0	272 6		268 0	253 0

Table III *Mean serum cholesterol (mg/100 ml) by number of cigarettes in daily cigarette smokers in relation to body weight (age groups 40–49)*

No of cigarettes		Body weight (kg) <70	70–79	80–89	90–99	100+	Total
1–4	*n*	114	203	127	44	13	501
	Chol	256 1	255 9	262 1	270 4	266 4	259 1
5–9	*n*	308	544	286	94	20	1 252
	Chol	262 7	269 7	276 2	278 1	268 0	270 0
10–14	*n*	566	854	544	150	44	2 158
	Chol	267 3	274 1	278 1	283 6	284 6	274 2
15–19	*n*	326	554	323	136	35	1 374
	Chol	263 1	271 4	278 3	275 1	294 2	272 0
20–24	*n*	207	354	285	120	41	1 007
	Chol	267 4	271 1	277 4	291 2	302 9	275 8
25+	*n*	80	145	122	63	21	431
	Chol	264 9	273 8	272 9	283 8	272 5	273 3
Unanswered	*n*	1	4	1	2	0	8
	Chol						
Total	*n*	1 602	2 658	1 688	609	174	6 731
	Chol	264 5	270 4	276 0	280 5	286 1	271 7

difference is statistically significant in the former age group ($p<0.05$) but not in the latter In filter versus non filter cigarettes the difference is statistically significant in both age groups ($p<0.01$)

In Table II the serum cholesterol/age trend is analyzed according to cigarette consumption Again at ages below 40 the numbers of men are too small to allow definite statistical conclusions In age groups 40–44 and 45–49 there is a statistically significant increase in the cholesterol value with increasing numbers of cigarettes ($p<0.01$) the most pronounced increase being found from consumption group 1–4 cigarettes to group 10–14 per day The cholesterol level for never cigarette smokers does not differ significantly from the 1–4 cigarette group ($p>0.10$)

Table III shows increasing cholesterol values with increasing body weight in daily cigarette smokers ($p<0.01$) This weight dependent cholesterol increase is present in all cigarette smokers irrespective of the daily amount of cigarettes Again cholesterol values increase with increasing numbers of cigarettes ($p<0.01$) This cigarette associated cholesterol increase is present in all weight classes

In age groups 40–44 and 45–49 (Table IV) body weight is lower in present-cigarette smokers than in never-cigarette smokers ($p<0.01$ in age group 45–49) In age group 40–44 body weight is slightly higher ($p>0.10$) and serum cholesterol lower ($p<0.01$) in non inhalers compared with inhalers In age group 45–49 these differences between inhalers and non inhalers are inconsistent and uncertain It should be noticed that those smoking 20 cigarettes a day or more in age group 40–44 have a higher mean body weight than both those smoking less and the never-cigarette smokers ($p<0.01$) Also in age group 45–49 the heavy cigarette smokers have a higher body weight than moderate smokers ($p<0.01$) while mean body weight in never cigarette smokers is higher than in smokers ($p<0.01$) In this age group only the 25+ cigarette smokers weigh significantly more than the never-cigarette smokers ($p<0.05$)

Also in ex-cigarette smokers body weight seems to be influenced by the number of cigarettes previously smoked the greater the earlier cigarette consumption the higher the body weight This observation is apparent in age group 40–44 as well as 45–49 (Table IV) ($p<0.01$) Also the serum cholesterol level tends to increase with an increasing number of cigarettes previously smoked ($p<0.01$)

Table V presents serum cholesterol body weight and height weight index in ex-cigarette smokers according to the time since they stopped smoking in 5 year age groups compared with values for present-cigarette smokers and never-cigarette smokers

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Table IV Mean serum cholesterol (mg/100 ml) body weight (kg) and height weight index (WI=H/∛w) by number of cigarettes per day in different groups of smokers in ex cigarette smokers and in never cigarette smokers (age groups 40–44 and 45–49)

No of cigarettes		Never cigarette smokers	Present cigarette smokers: Total	Inhalers	Non inhalers	Filter	Not filter	Ex cigarette smokers
Age group 40–44								
1–4	*n*		205	155	50	103	101	159
	Chol		254 8	255 1	254 1	255 2	254 3	248 4
	w		77 9	77 6	78 8	79 5	76 3	76 8
	WI		41 8	41 9	41 8	41 7	42 0	41 9
5–9	*n*		523	498	25	143	376	444
	Chol		269 1	269 4	263 9	266 2	270 3	260 4
	w		76 1	75 9	80 1	77 1	75 8	77 0
	WI		41 8	41 8	42 1	41 9	41 8	41 9
10–14	*n*		985	945	39	197	783	578
	Chol		270 1	270 3	263 2	263 1	271 8	259 7
	w		76 4	76 3	76 9	77 2	76 1	78 4
	WI		41 9	42 0	41 6	42 1	41 9	41 8
15–19	*n*		677	654	22	175	497	372
	Chol		269 3	269 6	265 1	265 1	270 7	265 6
	w		77 3	77 3	78 1	77 7	77 1	79 6
	WI		41 7	41 7	41 5	41 8	41 7	41 7
20–24	*n*		506	487	17	123	378	308
	Chol		273 3	273 4	272 2	274 9	273 2	265 1
	w		78 4	78 4	79 4	78 0	78 6	80 9
	WI		41 7	41 7	41 0	41 7	41 7	41 4
25+	*n*		213	197	16	55	157	203
	Chol		268 8	269 6	259 0	264 2	270 6	267 4
	w		79 6	79 6	79 7	80 5	79 2	83 3
	WI		41 7	41 7	41 0	41 5	41 7	41 1
Total	*n*	1 504	3 114	2 941	169	796	2 297	2 081
	Chol	255 0	269 2	269 7	261 4	265 0	270 8	261 4
	w	77 7	77 2	77 1	78 6	78 0	77 0	79 0
	WI	41 8	41 8	41 8	41 6	41 8	41 8	41 7
Age group 45–49								
1–4	*n*		299	246	53	110	185	207
	Chol		262 1	262 6	259 6	257 0	264 8	264 0
	w		76 7	76 9	75 9	76 8	76 8	76 5
	WI		41 7	41 7	41 8	41 8	41 7	41 7
5–9	*n*		742	689	52	169	567	614
	Chol		270 2	270 4	253 1	265 5	271 4	264 9
	w		75 9	75 9	77 7	78 0	75 3	77 4
	WI		41 8	41 9	41 5	41 8	41 9	41 8
10–14	*n*		1 191	1 143	48	206	973	734
	Chol		277 9	278 0	274 9	269 7	279 7	266 4
	w		76 1	76 1	75 7	77 0	75 9	77 9
	WI		41 8	41 8	41 3	41 7	41 8	41 6
15–19	*n*		703	665	36	160	536	364
	Chol		274 6	274 3	279 8	271 7	275 7	273.5
	w		77 2	77 0	80 0	79 6	76 5	79.5
	WI		41 7	41 8	41 2	41 5	41 8	41 4
20–24	*n*		508	487	20	103	404	374
	Chol		278 3	278 1	284 9	281 3	277 5	266 4
	w		78 5	78 7	74 1	78 7	78 4	80 6
	WI		41 5	41 5	41 2	41 4	41 5	41.5

Table IV *Cont*

No of cigarettes		Never cigarette smokers	Present-cigarette smokers: Total	Inhalers	Non inhalers	Filter	Not filter	Ex cigarette smokers
5+	*n*		223	211	11	70	153	222
	Chol		276 7	279 9	269 4	277 6	276 4	275 3
	w		80 1	79 8	85 4	80 2	80 0	82.2
	WI		41 4	41 4	40 8	41 4	41 4	41 1
Total	*n*	1 377	3 669	3 443	221	818	2 821	2 475
	Chol	265 1	274 4	274 6	271 0	269 7	275 7	267 7
	w	78 3	76 9	76 9	77 2	78 2	76 5	78 6
	WI	41 6	41 6	41 7	41 5	41 6	41 7	41 5

Only in age groups 40–44 and 45–49 are the numbers sufficient to allow conclusions There is a possible trend towards a lower serum cholesterol in relation to time since quitting ($p<0\ 05$) In ex-cigarette smokers as a whole body weight is higher than in present-cigarette smokers while the height weight index is the same which means that smokers are not leaner than ex-cigarette smokers This observation indicates that the weight gain usually observed when people stop smoking (and which applies in the present study only to the first year) seems to be temporary

Table V *Mean serum cholesterol (mg/100 ml) body weight (kg) and height weight index ($WI=H/\sqrt[3]{w}$) by age and time since cessation of smoking cigarettes Ex cigarette-smokers never-cigarette smokers and present-cigarette smokers*

Age group		Ex-cigarette-smokers (time since stopped): 3 mo	3 mo –1 y	1–5 y	5+ y	Total	Never cigarette smokers	Present cigarette smokers
–24	*n*	7	13	24	1	46	132	191
	Chol	192 8	205 6	205 8	–	203 4	195.3	201 5
	w	78 3	71 2	75 4	–	74.2	74 4	72 9
	WI	42 4	42 8	42 9	–	42 9	43 4	43 3
–29	*n*	10	24	50	17	101	215	249
	Chol	214 5	227 3	222 9	208 0	220 6	217.5	230 0
	w	76 5	75 8	74 6	72 9	74.8	75 8	75 2
	WI	42 3	42 6	42 7	42 6	42 6	42 6	42 5
–34	*n*	4	13	41	39	97	109	167
	Chol	241 2	266 5	237 1	246 6	245 0	232 8	247 8
	w	76 5	80 0	78 5	76 6	77 9	76 4	75 1
	WI	41 8	42 1	41 9	42 1	42 0	42 1	42.3
–39	*n*	6	10	30	43	90	100	181
	Chol	253 0	234 3	250 8	239 3	243 9	232 5	261 1
	w	78 8	82 6	80 0	79 5	79 9	76 4	77 6
	WI	41 7	41 9	41 7	42 1	41 9	42 1	41 9
–44	*n*	89	159	711	1 105	2 081	1 504	3 114
	Chol	260 1	269 0	260 6	261 1	261 3	255 0	269 2
	w	80 8	81 3	79 9	78 0	79 0	77 7	77.2
	WI	41 2	41 4	41 6	41 8	41 7	41 8	41 8
–49	*n*	91	152	718	1 506	2 475	1 377	3 669
	Chol	280 1	271 3	270 8	265.2	267 7	265 1	274 4
	w	78 3	80 2	78 9	78 4	78 6	78.3	76 9
	WI	41 5	41 5	41 6	41 7	41 7	41 6	41 7
Total	*n*	207	371	1 574	2 711	4 890	3 437	7 571
	Chol	263 8	264 0	262 4	262 4	262.5	251.8	268 1
	w	79 3	80 1	79 2	78.2	78 7	77 6	76 8
	WI	41 5	41 6	41 7	41 7	41 7	41 8	41.8

The serum cholesterol level in ex cigarette smokers lies between that of present-cigarette smokers and of never-cigarette smokers In age group 45–49 the mean cholesterol level in ex cigarette smokers is only insignificantly higher than in never cigarette smokers

DISCUSSION

A positive correlation between serum cholesterol and body weight has been demonstrated in many studies (5 13 14 17 18) However information is scarce about the interrelationship of serum cholesterol and smoking and body weight and smoking (2 8 13 16)

The present study reveals a significant positive correlation between serum cholesterol and the degree of exposure to cigarette smoke in the order never-cigarette smokers ex-cigarette smokers present non inhaling cigarette smokers present inhaling cigarette smokers and present non filter cigarette smokers Moreover in present cigarette smokers serum cholesterol increases with increasing daily number of cigarettes Such an increase in serum cholesterol with the degree of tobacco smoking does not seem to have been commented on before The reason for this might be that the simple correlation coefficient is used as an expression of dependence between risk variables However it should be noticed that this coefficient only measures the degree of linearity between the factors studied

A curvi linear relationship may have a low correlation coefficient but the factors may still be highly dependent Thus examining the relationship between serum cholesterol and number of cigarettes a curvi linear dependency seems to exist (Table II) but the simple correlation coefficient is low ($r =$ 0 054) as in other studies (18) However r differs significantly from zero because of the large number of individuals (=16 525) Also in the present study serum cholesterol tends to increase with increasing body weight

However cholesterol seems to increase with the number of cigarettes within each weight class indicating that the degree of exposure to cigarette smoke itself influences the cholesterol level independent of the body weight (Table III)

That heavy cigarette smokers tend to have higher serum cholesterol than never cigarette smokers has previously been shown (9 10) It has been suggested that elevated serum lipids in smokers may be a result of dietary differences because food preferences differ according to smoking habits (2) The lower cholesterol value in never-cigarette smokers may probably also be due to a selection of health conscious individuals in the never-cigarette smoking group who have also changed their dietary habits and physical activity compared with heavy cigarette smokers However overeating among cigarette smokers compared with never cigarette smokers is not the case as the average body weight—at least in the light and moderate cigarette smokers—was found to be lower than in never-cigarette smokers confirming previous observations (11) Still a qualitative difference in the food pattern between cigarette smokers and never cigarette smokers is of course possible

It has been shown that differences in metabolism exist between smokers and non smokers During the weeks following cessation of smoking an increase has been found in body weight together with decreases in oxygen consumption heart rate and protein bound iodine level in blood (1 4) If the inverse changes take place in cigarette smokers one would expect a lower body weight in cigarette smokers than in non cigarette smokers Thus it might be that the lower weight of cigarette smokers can be explained at least partly by metabolic changes inducing a weight reduction as usually seen This concept is supported by the findings (7) that both i v nicotine and simulated cigare[illegible] smoking in dogs significantly raised the total body oxygen consumption and the arterial concentration of free fatty acids Lastly a direct cholesterol increasing effect of tobacco smoking is also possible Inhalation of carbon monoxide in rabbits increases not only the lipid concentration in the aorta wall but also to some extent the serum cholesterol concentration (12)

An immediate and highly significant increase in cholesterol after a single cigarette has recently been reported lasting for 90 min in most of the test subjects (3) Whatever the explanation may be the present demonstration of a quantitative cigarette related increase in serum cholesterol does not seem to be as trivial as in earlier studies (10)

Body weight and serum cholesterol in ex-cigarette smokers show increasing values with increasing num[illegible] of cigarettes smoked before quitting smok[illegible] observation is not easily expla[illegible] [illegible]ons are that earlier

igarette smoking has caused persistent metabolic hanges or that dietary and other living habits per sist after cessation of smoking Finally, support rs of the constitutional hypothesis (15) would laim that those who are constitutionally deter mined to start smoking are the same individuals as hose who are determined to have a higher serum holesterol level and a higher body weight Of ourse such a biological trend can be modified by ietary changes (6) Serum cholesterol and body eight are both significantly lower in ex-cigarette mokers 5 years after quitting than in present-ciga rtte smokers Thus 5 years and more after dis ontinuing smoking the expected parallellism of erum cholesterol and body weight seems to be re stablished

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Serum Lipid and Lipoprotein Concentrations in Chronic Uremia

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ABSTRACT The concentrations of triglycerides and cholesterol have been determined in total serum and in the three major serum lipoprotein classes—very low (VLDL) low (LDL) and high (HDL) density lipoproteins—after an overnight fast in 39 patients with chronic uremia of more than 2 years' duration and with serum creatinine above 350 μmol/l The values were compared with data from healthy male and female controls The findings were similar for male and female uremics Hypertriglyceridemia was common while serum cholesterol tended to be normal or subnormal With the conventional typing system for hyperlipidemia types II A, III and IV were present in 6, 9 and 30%, respectively The triglyceride and cholesterol concentrations in VLDL were increased, while their normal relation for this lipoprotein class was maintained In LDL the concentration of triglycerides was increased while that of cholesterol was low The LDL composition therefore was changed to be more triglyceride rich than normal The changes in concentration and composition of LDL indicated that the levels of LDL_1 were raised and of LDL_2 decreased in chronic uremia Increased levels of LDL triglycerides occurred more frequently (40%) than increased levels of VLDL triglycerides (33%) The most striking and consistent lipoprotein abnormality was a low HDL cholesterol which was not related to high VLDL levels The HDL triglycerides, on the other hand tended to be somewhat high The importance of the raised levels of the triglyceride rich VLDL and LDL_1 and the decreased levels of HDL cholesterol for the rapid development of atherosclerotic vascular diseases which occur in chronic uremia is discussed It is of interest in this context that both total cholesterol and LDL cholesterol were low The possible mechanisms underlying the lipoprotein abnormalities in chronic uremia are discussed and it is suggested that they are complex

While the hyperlipidemia of the nephrotic syndrome has been well known for a long time the serum lipids in chronic uremia have been studied only during the last 5–10 years The reasons for the recent interest in serum lipids in chronic uremia are two-fold Firstly the techniques for long term dialysis in chronic uremia were not developed until the beginning of the 1960s Secondly it is now well known that patients with chronic uremia rapidly develop atherosclerotic diseases and about 60% of these patients die from cardiovascular complications (20 22 23) In view of the close association between various abnormalities of the serum lipids and atherosclerotic diseases it is important to study the serum lipids in chronic uremia Bagdade et al have in several recent studies (1 2 3) demonstrated the presence of moderate hypertriglyceridemia in patients with chronic uremia Similar findings have been observed by others (15 17 21)

It is understandable in view of the recent arousal of interest in lipid metabolism in chronic uremia that so far no detailed lipoprotein analyses are available in this condition We have therefore studied the cholesterol and triglyceride contents of the very low (VLDL) the low (LDL) and the high (HDL) density lipoproteins in a group of patients with chronic uremia in order to characterize the lipoprotein abnormalities occurring in this condition Previously unrecognized abnormalities were found in all of these three major lipoprotein classes

PATIENTS

Blood was taken in the morning after fasting overnight from 15 female (age 24–72 years mean 50) and 24 male

Table I *Concentrations (mmol/l) of triglycerides (TG) and cholesterol (Chol) in total serum and in the three lipoprotein classes*

	Total		VLDL		LDL		HDL	
	TG	Chol	TG	Chol	TG	Chol	TG	Chol
Males								
Control (mean)	1 80	6 40	1 00	0 52	0 52	4 20	0 24	1 37
Uremic								
Mean	2 18	5 17	1 17	0 83	0 69	3 18	0 26	1 07
±S E M	0 20	0 32	0 17	0 14	0 05	0 28	0 02	0 10
Females								
Control (mean)	1 40	6 71	0 67	0 34	0 48	4 17	0 27	1 79
Uremic								
Mean	2 06	5 53	1 18	0 72	0 63	3 58	0 21	1 06
±S E M	0 20	0 36	0 17	0 09	0 06	0 34	0 01	0 04

(age 22–82 years mean 48) patients with serum creatinine ranging from 354 to 1 609 μmol/l (mean for women 632 men 862) The duration of the chronic renal failure was more than two years The patients had fairly rigid and individualized instructions about their food intake from dieticians and were recommended diets containing on an average 33 56 and 11% of the calories from fat carbohydrate and protein respectively

The following kidney diagnoses were present (no of females/no of males) kidney dysplasia 4/4 cystic kidney disease 2/4 pyelonephritis 3/4 glomerulonephritis 1/4 nephrocalcinosis 1/2 polyarteritic kidneys 2/0 diabetic kidney 1/0 unknown cause of renal insufficiency 1/6 Nephrotic syndrome was excluded on the basis of an analysis of urinary protein which was below 2 5 g/day in all patients except 2 who had amyloidosis of the kidney These two patients had serum albumin concentrations of 27 and 29 g/l but no edema The serum albumin concentration ranged from 27 to 41 g/l

Four patients were on steroids two on cortisone acetate 37 5 mg/day one patient received prednisolone mg/day and one dexamethasone 12 mg/day None had immunosuppressive treatment Five patients were treated with allopurinol Almost all patients were given aluminium hydroxide Five women and four men were on chronic hemodialysis All patients except one who had lost 10 kg during the last 4 months had been weight stable during the preceding 6 months All consecutive patients with chronic uremia attending the Kidney Unit of the Department of Medicine between Nov 1974 and Jan 1976 (except one with malignant disease) have been included

METHODS

Blood was allowed to clot at room temperature for 1–2 hours serum was then recovered after low speed centrifugation and added with 5% Na EDTA to a final concentration of 0 05% Serum was then centrifuged at density 1 006 in a 40 3 rotor in a Beckman model L ultracentrifuge at 40 000 rpm for 16 hours The top two ml were harvested quantitatively and are termed the VLDL fraction The infranatant was added with $MgCl_2$-heparin which quantitatively precipitates LDL leaving HDL in solution (6 7) Cholesterol (5) and triglycerides (18) were determined with semiautomated techniques on a Technicon AutoAnalyzer Model I after isopropanol extraction of whole serum the VLDL fraction the infranatant after ultracentrifugation (containing LDL+HDL) and of the supernatant (HDL) after precipitation Values for LDL were then calculated by difference A detailed description of the method for lipoprotein analysis is given elsewhere (7) The average recovery of triglycerides in the three lipoprotein classes was 97% (range 85–116) The corresponding figures for cholesterol were 97 and 90–105 Agarose gel lipoprotein electrophoresis according to Noble (25) was performed on whole serum and on the top (VLDL) and the bottom (LDL+HDL) fraction after ultracentrifugation (7)

The lipoprotein data of the patients have been compared with a group of healthy control mal es from Uppsala (12) analysed by the same for the lipid determinations which were Analyzer Model II (27) That method g

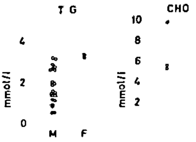


Fig 1 Individual values f tions of triglycerides (TG) male (M) and female (F) pa the 10th 50th (median) a controls (12)

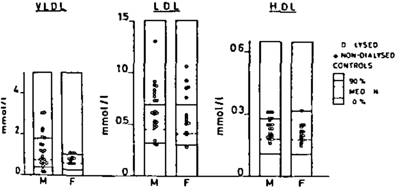


Fig 2 Individual values for the concentrations of triglycerides in VLDL LDL and HDL in the male (*M*) and female (*F*) patients with chronic uremia Control values as in Fig 1

are identical with the present for cholesterol but 0 2 mmol/l higher for total and LDL triglycerides (unpublished data) Types of hyperlipoproteinemia were defined as follows Type II A and type IV elevation (above 90th percentile) of only LDL cholesterol or only VLDL triglycerides respectively Type III elevated VLDL presence of a late pre β lipoprotein (8) and an elevated cholesterol/triglyceride ratio in VLDL

RESULTS

The mean values for triglyceride and cholesterol concentrations are given in Table I As seen in earlier studies the total triglycerides were higher in patients with chronic uremia than in controls Total cholesterol however was lower in the patients Fig 1 shows the individual data for total triglycerides and cholesterol All patients but 6 males and one female (82%) had triglyceride values *above* the median for the controls The situation was the opposite for cholesterol all but 4 males and 3 females (82%) had values *below* the median value

In all lipoprotein classes the triglyceride concentrations in the patients were mainly distributed above the median values of the controls (Fig 2)

The tendency towards raised lipoprotein triglyceride content was most pronounced in LDL

The distribution of cholesterol in the lipoprotein classes (Fig 3) behaved quite differently from that of triglycerides VLDL cholesterol had a pattern similar to VLDL triglycerides with raised levels On the other hand the cholesterol content of LDL and still more of HDL was remarkably low in both the male and the female patients

The combined electrophoretic and ultracentrifugal analysis revealed the presence of type III hyperlipoproteinemia in two patients Type II A and type IV hyperlipoproteinemia were present in 3 and 11 cases respectively

Since our previous studies have shown a very close relation between the cholesterol and triglyceride contents of VLDL this relation was studied in the present material and found to be very close to that seen previously (12 26) The average cholesterol/triglyceride ratio was 0 71 for the male and 0 61 for the female patients

A close linear relation also exists between the cholesterol and triglyceride contents of LDL (12 26) This relation was however markedly changed



Fig 3 Individual values for the concentrations of cholesterol in VLDL LDL and HDL in the male (*M*) and female (*F*) patients with chronic uremia Control values as in Fig 1

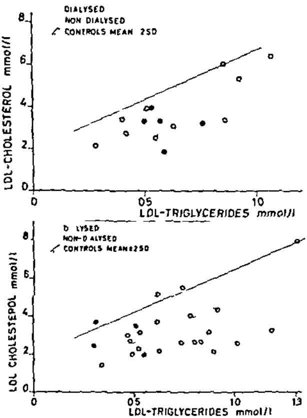


Fig 4 Individual values for the concentrations of cholesterol and triglycerides in LDL in the female (*top*) and the male (*bottom*) uremic patients and the relation between cholesterol and triglycerides in LDL for controls (12)

for the uremic patients (Fig 4) Their LDL par were much more triglyceride rich in rela to their cholesterol content than the normal DI *from the controls*

DISCUSSION

In this material as in others (1 2 3 15 17 21) with chronic uremia hypertriglyceridemia was common The lipoprotein analysis revealed that the content of triglycerides was elevated in both VLDL and LDL The elevation of LDL triglycerides was the most frequent lipoprotein triglyceride abnormality and its possible cause will be discussed below

The quantitative lipoprotein analysis furthermore revealed that there are more lipoprotein abnormalities in chronic uremia than hypertriglyceridemia *The most striking one was the subnormal HDL* cholesterol concentration Since HDL cholesterol constitutes such a small part of total serum cholesterol—around 20%—pronounced reductions of this lipoprotein class will give rise to only a minor fall in total cholesterol and therefore quantitative lipoprotein analysis is necessary in order to detect reductions of HDL cholesterol Also LDL cholesterol was often low About 80% of the uremic patients had values below the median value for LDL cholesterol in the controls

Although these quantitative lipoprotein analyses provide a much more detailed picture of the disturbances of lipid transport in uremic patients than the *simple measurements of total triglycerides and* cholesterol they give only a static picture at one point in time It is however possible from the present data on the composition of the lipoproteins to formulate some hypotheses about the possible mechanisms of deranged lipid transport in chronic uremia The formulation of such a *hypothesis* is based upon the present concepts of the interrelation and metabolism of VLDL and LDL as schematically depicted in Fig 5 In essence the liver secretes triglyceride rich lipoproteins—VLDL—into the blood (step 1 Fig 5) Triglycerides are then split off continuously from the lipoprotein molecules (steps 2, 3 and 4 Fig 5) initially at least by the action of lipoprotein lipase Through these various steps lipoproteins of increasing density being enriched in cholesterol are formed The last product formed in this lipolytic chain—LDL_2—is then catabolized Although the subfractions of VLDL and LDL were not measured here the

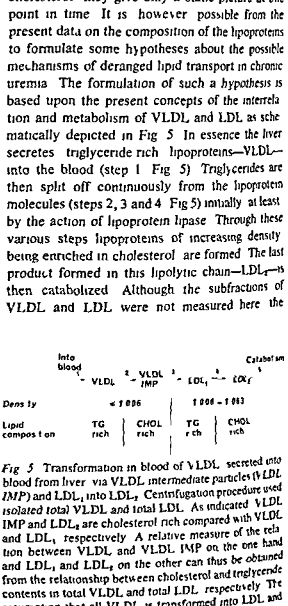
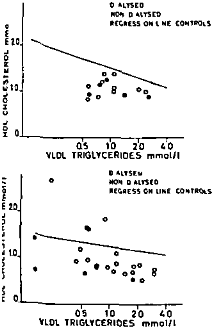


Fig 5 Transformation in blood of VLDL secreted into blood from liver via VLDL intermediate particles (*VLDL IMP*) and LDL_1 into LDL_2 Centrifugation procedure used *isolated total* VLDL *and* total LDL As indicated VLDL IMP and LDL_2 are cholesterol rich compared with VLDL and LDL_1 respectively A relative measure of the relation between VLDL and VLDL IMP on the one hand and LDL_1 and LDL_2 on the other can thus be obtained from the relationship between cholesterol and triglyceride contents in total VLDL and total LDL respectively The assumption that all VLDL is transformed into LDL and that LDL is derived *solely from* VLDL has not been validated for all types of hyperlipidemia in man



'g 6 Individual values for the concentrations of HDL holesterol and VLDL triglycerides in the female (*top*) nd the male (*bottom*) uremic patients and the significant egative relation between these two lipoprotein para eters in controls (normolipidemic+primary hyper ›demia (26))

ieasurement of both cholesterol and triglycerides ives an indication of the relative proportions of the 'LDL subclasses as discussed in more detail else here (9 26) The VLDL data with a normal holesterol/triglyceride ratio indicate that there as no generally occurring block of step 3 as e g ı type III hyperlipoproteinemia The raised proortion as well as amount of triglycerides and the w absolute amount of cholesterol in LDL indi ates that LDL_1 was increased and LDL_2 de eased It is not possible to explain the lipoproin abnormalities in chronic uremia—increase in LDL and LDL_1 but decrease in LDL_2—by a ange of only one of steps 1–5 in Fig 5 One netic explanation for these lipoprotein abnor alities would be a decrease in step 1 combined th a more pronounced decrease in the lipolytic ages 2 3 and 4 with a normal rate of step 5 Or metabolic terms a decreased synthesis of VLDL mbined with a decrease *in the lipolytic (lipo*otein lipase) activities or in the later handling of e liberated fatty acids i e the fatty acid incor ration into adipose tissue (14) There are of urse several other kinetic possibilities which might explain the VLDL and LDL abnormalities Another would for instance be an increase in steps 1 and 5 Further studies of the turnover of VLDL and LDL are necessary to disclose the cause of the derangements of VLDL and LDL in chronic uremia

With regard to the low HDL cholesterol levels we cannot offer any explanation concerning the possi bilities of either a decreased synthesis or increased catabolism It is known however that in pri mary hypertriglyceridemias HDL cholesterol de creases with increasing VLDL triglycerides (26) We have analysed this possibility in Fig 6 which shows that the HDL cholesterol is lower in the uremic patients than in non uremic subjects at one and the same VLDL triglyceride level The usual association between low HDL and raised VLDL is thus not present Therefore other mechanisms causing low HDL than those present n primary hypertriglyceridemias are probably op rating in chronic uremia

There was a positive correlation between the concentration of serum albumin and HDL cholesterol (*r*−0 43 *for men r*−0 56 *for women both* significant at the 5% level) A decreased synthesis of these two serum proteins due to liver insuffi ciency or to low protein intake is a possibility

Another aspect of the low HDL levels in chronic uremia is that in view of the importance of HDL as carriers of the apolipoproteins necessary for the lipolytic breakdown of VLDL to LDL_2 (19) a low HDL level may be of primary importance for the raised levels of the triglyceride rich VLDL and LDL_1

The present lipoprotein findings—increase in tri glyceride rich VLDL and LDL_1 and decrease in LDL_2 and HDL—are of interest in relation to the rapid development of cardiovascular diseases in chronic uremia It is noteworthy in this connection that the concentration of the atherogenic cholesterol rich LDL_2 was low However there is in creasing evidence that even hypertriglyceridemia (4 10 16) and raised levels of VLDL (11 13) are associated with ischemic heart disease In addition it may be of particular relevance that we recently observed that increases in LDL triglyceride were *quite common in ischemic heart disease* (13) It is of further interest that a low level of HDL has recently been claimed to be of importance for ischemic vascular disease (13 24) It is possible that the occurrence in chronic uremia of the com

bination of the three lipoprotein abnormalities 1) increased VLDL, 2) increased LDL_1 and 3) decreased HDL may be particularly malignant and a major contributing factor for the rapid development of atherosclerotic vascular disease in this condition.

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Blood Levels of 3,5,3'-Triiodothyronine and Thyroxine Differences between Children, Adults, and Elderly Subjects

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ABSTRACT The serum levels of 3 5,3 triiodothyronine (T_3) and thyroxine (T_4) in children, adolescents adults, and elderly subjects have been measured by radioimmunoassays It was found that while the T_4 levels were essentially equal in all age groups examined, the T_3 levels were markedly different In children and adolescents (1–15 years) high values were recorded indeed, they exceeded the upper normal limit in adults (20–80 years) From the age of 20, the T_3 levels remained unaltered until the age of 80 after which there was a further reduction, to values approaching the lower normal limit for T_3 in middle aged subjects The findings emphasize that separate normal values must be established for different age groups, in order to avoid diagnostic misinterpretations and therapeutic failures

The development of radioimmunoassays for determination of the blood concentrations of 3 5 3 triiodothyronine (T_3) and thyroxine (T_4) has contributed much to the knowledge of thyroid function The assays are increasingly used for diagnostic purposes and they may also be of value for monitoring substitution therapy with thyroid hormone For an adequate use of the assays reliable normal values must be established and possible physiologic variations should therefore be evaluated It has been reported from various laboratories that while the T_4 concentrations in serum display virtually no reduction with increasing age those of T_3 show a decline in old age (3 7 9) Low levels of T_3 and T_4 have also been recorded in neonates Shortly after birth however the levels seem to increase markedly as high concentrations in serum of infants (1 4 6)

The present investigation demonstrates that after the neonatal period children have high serum T_3 concentrations and that the T_3 levels remain high till the age of 15 Indeed the values often exceed the upper normal limit for T_3 in adults In addition the study confirms that there is a reduction of serum T_3 *in the very old In contrast to T_3 T_4 levels re*main unaltered during the whole life span The recorded T_3 differences are large enough to have diagnostic and therapeutic consequences

MATERIAL AND METHODS

Blood samples were drawn by vein puncture from 167 patients admitted to the Department of Surgery University of Lund because of minor surgical complaints None of the subjects were fasted before blood sampling This is emphasized as fasting causes a rapid fall in serum T_3 concentrations (8 12) The subjects were grouped by age as specified under Results In each case the absence of thyroid hyper or hypofunction was judged clinically by the same doctor Serum was prepared by centrifugation at room temperature and was stored frozen below −20°C until assayed

The serum concentrations of T_3 and T_4 were assessed by radioimmunoassays (3 5) in triplicate (T_3) or duplicate (T_4)

RESULTS

Fig 1 demonstrates the mean serum concentrations (±2 S D) of T_3 and T_4 in clinically euthyroid subjects of different ages It appeared that the T_4 concentrations were almost equal in all groups but that the T_3 concentrations differed markedly

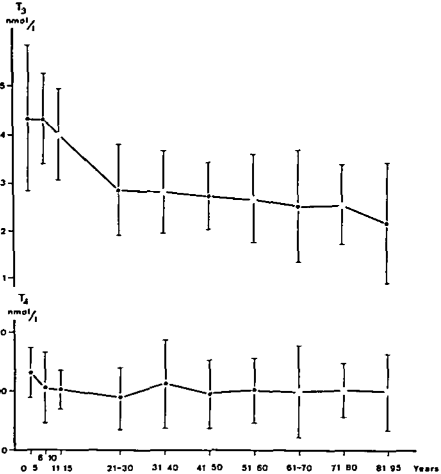


Fig 1 Mean values (±2 S D) of T_3 and T_4 concentrations in serum from non fasted euthyroid human subjects of different ages ($N=167$)

tion of the T_3 levels after the age of 15 The mean T_3 value for the total group of children (1–15 years) was significantly ($p<0\ 001$) higher than that of adults (20–80 years) Indeed the mean T_3 level in children was so high that it exceeded the upper normal limit for serum T_3 in adults (3 2 nmol/l)

Between the ages of 20 and 80 the T_3 levels seemed to remain unaltered but after 80 there appeared to be a further reduction In several subjects over 80 the T_3 values were below the lower normal limit for adults (1 5 nmol/l)

No difference in the T_3 concentrations was recorded between males and females within any of the age groups in the material as a whole

DISCUSSION

Various studies (1 4 6) have shown that T_3 concentrations are low in newborn children These reports infer that this situation is drastically altered shortly after birth as the serum T_3 concentrations in infants are high (1 4 6) The present investigation indicates that the T_3 concentrations then remain elevated throughout childhood as children aged 15 were found to have high serum T_3 concentrations In addition to the elevated T_3 concentrations in infants (1, 4, 6) high serum T_3 levels have previously been recorded in 5–9 year-old children (9) It is claimed in another study (10) that serum T_3 in children is equal to that in adults but these subjects were fasted before blood sampling and fasting has been shown to reduce serum T_3 (8 1?) In the present study, where fasting conditions were avoided serum T_3 levels were so high that they exceeded the upper normal limit for T_3 in adults During late adolescence the T_3 levels apparently fall but between the ages of 20 and 70–80 they seem to remain unaltered After that a further reduction apparently occurs in keeping with previous reports (3 7 9) Thus during a normal human life span serum T_3 is low at the time of birth increases markedly during early infancy remains high during childhood is reduced after adolescence then remains stable until late middle age and ultimately decreases in old age

In contrast to the concentrations of serum T

those of T_4 appear to remain essentially unaltered throughout life an observation which agrees with previous studies (7)

As the proportion of T_4 bound to serum proteins is greater than that of T_3 it is not very likely that the age related variation of the T_3 concentrations is secondary to age related differences in the serum concentrations of TBPA or TBG Consequently the T_3 alteration is probably caused by changes in the production of the hormone

As T_3 in serum arises both from direct thyroidal secretion and from extrathyroidal deiodination of T_4 (2 9 11 13) both an age related change of thyroid activity and an age related alteration of the peripheral conversion of T_4 to T_3 could contribute to the inverse relation between serum T_3 concentrations and age The present data cannot indicate whether either or both mechanisms are responsible Irrespective of their cause however the age related T_3 differences are large enough to have diagnostic and therapeutic consequences To exemplify the overall use of normal T_3 values derived solely from samples in healthy adults could lead to misinterpretation of children s physiological T_3 concentrations as reflecting hyperthyroidism and the control of T_4 replacement therapy by measurements of serum T_3 could lead to insufficient substitution doses in hypothyroid children At the other end of the spectrum in old people mild hyperthyroidism could remain undetected

In conclusion clinical laboratories should establish normal values for different age groups and the importance of adequate clinical judgements in the diagnosis and therapeutic control of thyroid diseases must be emphasized

ACKNOWLEDGEMENT

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On the Influence of Concomitant Food Intake on Sulfonamide Bioavailability

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BSTRACT The influence of food intake on the bio-ailability of a frequently used short acting sulfon ide sulfaisodimidine (Elkosin®), has been ex mined in eight healthy volunteers The drug was ad inistered as a single oral dose, both on an empty omach and together with a standardized breakfast imerous venous blood samples were drawn for the st eight hours after ingestion of the drug, and e concentration of unmetabolized sulfonamide in rum was assessed by spectrophotometry The ob rvations indicate that concomitant food intake al rs neither absorption rate peak concentration ne to reach peak concentration, elimination rate, r total amount of sulfonamide reaching the general rculation Thus, the absorption of orally admin tered sulfaisodimidine is not at all affected by con mitant intake of food This finding contrasts with evious observations on some other sulfonamides, id it may signify a therapeutic advantage of sulfa odimidine In addition, the amount absorbed owed only a little interindividual variation This ggests that the use of standardized size and interval sulfaisodimidine dosage can be recommended The esent findings emphasize that conclusions about the orption of a certain drug should not be derived n studies with other, albeit chemically related, mpounds

h in primary and in hospital care a common estion is whether a drug prescribed for oral ad in stration can be ingested together with food or ould be taken on an empty stomach The question ten remains unanswered however as informa on on the influence of food intake on the gastro testinal absorption of drugs is sparse Therefore have initiated a series of investigations concern g the influence of food intake on the bioavail bil ty of various drugs

In a preceding report it was demonstrated that the bioavailability of isoniazid (INH) is drastically reduced by concomitant food intake (3) a finding of importance in the treatment of tuberculosis Sulfon amides bear some chemical and pharmacologic re *semblance to INH They have a bacteriostatic ca* pacity and this is related to a free and reactive amino group in the molecule (7) Acetylation of the amino group not only affects the bacteriostatic capacity but is also part of the elimination process for these drugs (6 7) However the degree of acet ylation varies markedly between different sulfon amides some being extensively acetyla ed while others are mainly excreted untransformed (7) In addition the degree of absorption and the affinity to proteins differ among sulfonamides Hence sulfonamide kinetics can show major differences between different preparations

It has been reported that the gastrointestinal ab sorption of sulfonamides is delayed by concomitant food intake (2 5) However in view of the varied kinetics of different sulfonamides this finding need not signify that food intake influences the absorp tion of all sulfonamides in a similar fashion or to a similar degree In the present study we investigated the possible influence of concomitant food intake on the gastrointestinal absorption of one of the most frequently used sulfonamides the short acting sulfaisodimidine (Elkosin®) It was found that nei ther the rate nor the degree of absorption of this sulfonamide was influenced by food intake

MATERIAL AND METHODS

Eight clinically healthy volunteers 4 females and 4 males aged 18–35 body weight 55–75 served as test subjects Routine blood status was normal in all After

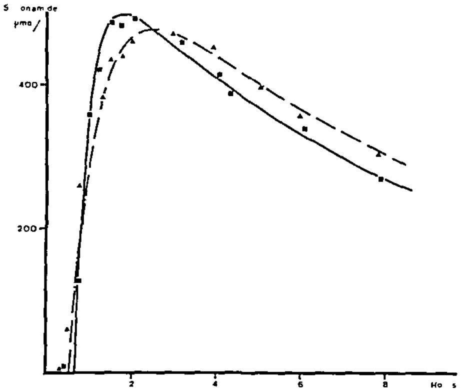


Fig 1 Calculated serum levels (μmol/l) of unmetabolized sulfonamide in one of e ght volunteers given a single oral dose of sulfaisodimidine (2 g) on an emp y stomach (—) and together w th a standard zed breakfast ( ) There was virtually no difference between the pre and post prand al curves either in this or in the other 7 subjects (Table I)

tion from food and liquid for 10 hours (10 p m – 8 a m) the subject got a polyethene cannula inserted into an ante brachial vein and 10 ml of blood was collected (0 value—blank) Thereafter sulfaisod m dine 2 g (4×0 5 g tablets all of the same brand and bat h Elkosin® CIBA-GEIGY Basle Switzerland) was ingested either together with 100 ml of drinking water or immediately after a standard ized breakfast The breakfast prepared by a d etician was composed of 150 ml low fat milk 100 ml orange juice 1 egg 2 p eces of crisp bread 5 g margarine 20 g orange marmalade and 20 g cheese This equals 20 g (20%) 17 g (35%) fat and 50 g (45%) carbohydrates and total energy of 1840 kJ (440 kcal) About 100 ml non black coffee was included The nurse collect the blood samples supervised eating and ingestion of tablets When the tablets were taken on an empty stom ach the subjects abstained from food and liquid for an other two hours after drug administration

Blood samples (about 10 ml) were drawn before (0 hour) and at about 15 30 45 60 75 90 105 120 180 240 300 360 and 480 min after drug ingest on The exact time (adjusted to the nearest min) of blood sampl ng (when the sampling tube was half-filled) was recorded and used in calculations Before each blood sampling 1 2 ml blood was drawn and d scarded and after each sampling 2–4 ml 0 15 M saline was injected via the cannula. The blood samples were left at room temperature for more than one but less than two hours They were then centrifuged and serum was collected and frozen at 20°C until assessed for the content of unmetabolized sulfonamide For these measurements a spectrophotometric techn que (4) was employed

In pilot studies it was found that for orally adm nistered sulfaisodimidine for which a time lag for the onset of bulk influx has to be allowed for the kinetics could be de scribed very satisfactorily by a first order model for influx into and efflux from a single compartment According to this model the ideal time–serum concentration curve would obey the following equation

$$\text{serum drug concentration} = y \quad a\frac{k}{k_2-k_1}(e^{-k} \; -e^{-k_2 t}) \qquad (1)$$

where k_1 = the influx (invas on absorption) constant k_2 = the elimination (efflux) constant a = a factor proportional to the given dose (the dose divided by the individual volume of d stribution for the drug) The usual procedure was to prepare a plot of drug serum concentrations versus time *the experimental plot* The elimination constant k_2 was then estimated from repeated regress on and correlation analyses of ln y on t (t = time in minutes) of the descending part of the experimental plot The set of points showing the highest linear correlation (at least three points) was used for the estimate of k_2 Both the time lag (t_l) for the onset of influx of the bulk of the drug and the time of maximal serum drug level (t_m) were estimated from the experimental plot The d fference t_m $t = t$ was used together with k_2 to obtain k_1 by numerical analysis to at least four s gnif cant d gits

The area under the experimental plot from t at which value y was taken as zero to $t_{last\ sampling}$ which in these experiments was around 480 min was computed as the sum of successive trapezo ds and called ∫exp For the same interval ∫calc the integral of the ideal curve according to eq 1 was computed on the assumption that $a = 1$ The correct a for fitting the ideal curve to the experimental plot based on the same areas under the curves is

Table I *Kinetic parameters of sulfaisodimidine in eight healthy volunteers given a single oral dos on an empty stomach and together with a standardized breakfast*

t_{max} = time of peak concentration k_1 = influx (absorption) constant k_2 = elimination constant $t_{1/2}$ = elimination half life AUC = area under the serum concentration curve

Subj no	Estimated absorption delay (min)	t_{max} (min)	k_1	Absorption half life (min)	Calculated peak concentration (µmol/l)	k_2	$t_{1/2}$ (min)	Empirical AUC	Mean concentration (µmol/l)
Fasting									
1	30	150	0 02441	28	478	0 0015768	440	182 340	340
2	30	150	0 02596	27	334	0 001355	512	1[illegible]9 947	[illegible]70
3	15	150	0 02101	33	406	0 0015115	459	157 559	[illegible]8
4	25	85	0 05891	12	479	0 0019294	359	164 66	[illegible]
5	50	105	0 07638	9	368	0 0011292	614	142 331	
6	20	75	0 07082	10	420	0 0015706	441	151 779	[illegible]
7	20	130	0 02523	28	396	0 0019480	35[illegible]	150 679	
8	10	105	0 03035	23	412	0 0020669	335	140 876	[illegible]
Non-fasting									
	40	110	0 05387	13	499	0 0016258	426	177 053	[illegible]
	20	90	0 05467	13	370	0 0015571	445	135 138	[illegible]
	40	90	0 07289	10	489	0 0021177	327	161 068	33[illegible]
	40	110	0 04797	15	481	0 0020128	344	16[illegible] 631	[illegible]45
	30	250	0 00865	80	370	0 0020034	34[illegible]	144 997	30[illegible]
	15	90	0 04786	15	448	0 0015554	446	164 84[illegible]	[illegible]
	80	220	0 01908	36	441	0 0016666	416	141 096	[illegible]
	15	105	0 03359	21	368	0 0019479	356	157 349	3[illegible]
Statistical significance (F tests) of difference between fasting and non fasting conditions			N S		N S	N S	N S	N S	N S

$$= \frac{\int exp}{\int calc}$$

be used together with k_1 and k_2 to obtain *the ideal plot* hich is then superimposed on the experimental plot The calculated y value at t_1+480 min is used as the last int on the experimental plot in order to achieve com nsons based on an 8 hour mean serum level of the drug tween or within individuals

Differences were judged by the F test

RESULTS

each of the eight subjects the preprandial curve d the postprandial curve seemed to be almost ntica (Fig 1) and calculations on paired ob vation revealed no significant differences her between pre and postprandial absorption es peak concentrations mean concentrations JC (area under the serum concentration curve) ues or elimination rates (Table I) In addition interindividual differences appeared to be small ble I)

DISCUSSION

Earlier reports state that the absorption of sulfonamides is delayed by concomitant intake of food as judged from investigations on six different sulfonamides namely sulfanilamide sulfadiazine (5) sulfisoxazole sulfadimethoxine sulfamethoxypyridazine and sulfasymazine (2) This contrasts with the present observation that the absorption of sulfaisodimidine is completely unaffected by concomitant intake of food

In view of the variety of food ingredients in the breakfast given in the present study it is not likely that the divergence between this and the previous studies is due to differences in the breakfast composition Hence the most probable explanation is that in contrast to the other preparations the physicochemical and biopharmaceutical properties of sulfaisodimidine as Elkosin tablets are such that food intake neither directly nor indirectly affects the rate and degree of dissolution and absorption of this drug This is an apparent therapeutic advantage as tablets prescribed more than b i d are

liable to be ingested in close relation to meals However the advantage is mainly the achievement of a more rapid effect as food intake does not seem to reduce but only delay the absorption of other sulfonamides tested (2 5)

An additional finding was that the amount of sulfaisodimidine absorbed showed only a little interindividual variation This contrasts with observations on several other drugs e g isoniazid (3), and suggests that the use of standardized size and interval of sulfaisodimidine dosage can be recommended Irrespective of the clinical significance as to the use of sulfonamides the present findings emphasize that conclusions about the absorption of a certain drug should not be derived from studies with other albeit chemically related compounds

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The Renin–Aldosterone System and Renal Hemodynamics in Patients with Posttransplant Hypertension

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BSTRACT Plasma renin concentration (PRC), lasma aldosterone concentration (PAC), renal lasma flow (RPF) and glomerular filtration rate GFR) have been studied in 19 patients who had eceived a renal allotransplant Group 1 consisted of normotensive and group 2 of 12 hypertensive pa ents Bilateral nephrectomy was performed in all atients all were on a fixed daily sodium intake, and o antihypertensive agents were given No significant ifferences were found between the groups in age, me after transplantation or dosages of prednisone RC and PAC were normal in all but one patient group 1 and two in group 2 In these three atients a slight elevation of PRC was measured fter one hour in the erect position, a significant crease was measured in PAC, but not in PRC in oth groups After 6 days on a 10 mEq sodium iet, PRC and PAC increased significantly in both roups After a further 6 days on the diet plus 150 Eq sodium daily, significant decreases in PRC and AC were measured in both groups No differences ere detected in PRC or PAC between groups 1 and either before or after the two dietary periods PF was significantly lower in the hypertensive roup whereas no significant difference was found GFR between the groups No significant relation p could be demonstrated between blood pressure P) and PRC or PAC, and PRC and PAC were t correlated to each other RPF was significantly rrelated to mean BP and PRC in the normoten e group but not in the hypertensive It is concluded at PRC and PAC are normal in most patients with sttransplant hypertension whereas the RPF is creased It is suggested that an abnormal regula n of renin secretion plays a role in the sustained vation of BP after renal allotransplantation

rmanent elevation of blood pressure is a rather quent complication following renal allotrans antation (3 7 20) According to earlier studies some of the most important etiologic facto i development of posttransplant hypertension are renal artery stenosis (12 19) the presen e o the recipients own kidneys (3 12 14 2) corti costeroid therapy (27) recurrent g nerulone phritis (12) acute (23) or chronic rejecti n (29)

The role of the renin–aldosterone sy tem a d the changes in renal blood flow in p sttran p in hypertension are much disputed Plasma renin ac tivity (PRA) is elevated during acute rejec ion episodes (11 17 24 30 32) When the graft function was stable PRA was norma according to several reports (2 21 26 27) though one study (1) showed it to be elevated Sampso et al (26 27) have postulated that hypertension following renal allotransplantation is caused by mineralo-corticoid excess Cooke et al (4) however found a normal plasma aldosterone concentration (PAC) in transplanted patients Renal blood flow was found to be decreased in posttransplant hyperten sive patients tested with the Xenon or Crypton wash-out technique (1 10) but normal when meas urements were performed with clearance tech nique (1)

The cause of these conflicting results may lie in the heterogeneity of the patients studied The re cipients own kidneys were present in some pa tients the daily sodium intake varied and some patients received antihypertensive treatment when the studies were performed

In the present study we have investigated a normotensive and a hypertensive group of trans planted patients Bilateral nephrectomy had been performed well in advance of the studies in all the sodium intake was fixed during the study period and all drugs except immunosuppressive agents had been withdrawn for several wee

Table I *Clinical data on the patients investigated*

Pat no	Age (y)	Sex	Time after transplantation (mo)	BP (mmHg)	Creatinine clearance (ml/min)	Prednisone (mg/d)	Azathioprine (mg/d)	Hypertension before transplantation
Group 1								
1	50	♂	49	122/83	82	7.5	100	+
2	53	♂	75	128/83	74	0.0	175	+
3	39	♀	94	107/78	132	2.5	125	+
4	49	♀	108	122/73	97	1.25	100	0
5	47	♀	7	120/80	50	17.5	75	+
6	57	♀	22	138/80	92	10.0	125	+
7	61	♂	54	178/92	62	5.0	150	+
Group 2								
8	38	♀	50	135/103	50	15.0	100	0
9	37	♀	22	167/107	92	5.0	150	+
10	55	♀	30	177/115	136	5.0	125	+
11	54	♀	64	160/103	89	5.0	25	0
12	44	♀	25	168/107	67	10.0	100	+
13	29	♂	101	138/105	88	1.25	150	+
14	64	♀	15	187/120	93	10.0	125	0
15	58	♀	13	185/117	57	10.0	75	+
16	33	♂	31	160/100	65	10.0	125	+
17	26	♂	9	172/112	40	17.5	100	0
18	52	♀	26	220/100	12	15.0	75	+
19	36	♂	12	212/105	49	10.0	125	+

purpose of the present investigation was to compare the renin–aldosterone system and the renal blood flow in the two groups and to determine whether any relationship could be demonstrated between BP and plasma renin concentration (PRC), PAC or renal hemodynamics.

MATERIAL

Subjects

Nineteen transplanted patients were examined. Group 1 (nos 1–7) consisted of 7 normotensive patients, 3 males and 4 females, average age 51±7 years. Group 2 (nos 8–19) consisted of 12 hypertensive patients, 4 males and 8 females, average age 44±12 years.

Permanent posttransplant hypertension was defined as diastolic BP at or above 100 mmHg at the last three consecutive visits to our Out-patient Clinic.

The elevation of BP appeared within the first 5 months after transplantation in all but one patient (no 8), whose BP increased in association with a recurrent glomerulonephritis 14 months after transplantation. As shown in Table I, BP was 131/81±23/6 mmHg in group 1 and 173/108±26/7 mmHg in group 2. In all but one patient (no 18) the function of the graft had been stable for several months, and when the investigations were performed the creatinine clearance was 84±27 ml/min in group 1 and 70±32 ml/min in group 2. Heavy proteinuria (8–10 g/day) was present in two patients (nos 7, 19). None of the other patients had proteinuria. Two of the hypertensive patients had retinal changes corresponding to grade 1 and 9 to grade 2 (Keith Wagener). None had grade 3 or 4 changes. One patient (no 19) had proliferative diabetic retinal lesions. The cardiac thoracic ratio was increased in 6 patients, whereas 8 had ECG signs of left ventricular hypertrophy and/or strain. None had cardiac failure. I.v. urography was performed in all patients and none had signs of urinary obstructive diseases. Serum calcium was normal in all. Nine patients had previously been treated with α-methyldopa, cyclopenthiazide, alprenolol or guanethidine. All drugs except prednisone and azathioprine were withdrawn at least 4 weeks before the investigations in all but one patient (no 19), who continued with insulin.

The study took place 59±37 months after transplantation for group 1 and 33±27 months after transplantation for group 2. The prednisone dosages for group 1 were 6.25±6.1 and for group 2 9.48±4.8 mg/day and the azathioprine dosages 121±34 and 106±36 mg/day, respectively. There were no significant differences between the groups in terms of age, time after transplantation, incidence of pretransplant hypertension and dosages of prednisone and azathioprine (p>0.05).

Three patients (nos 2, 3, 13) received an allograft from a relative, whereas the others received cadaveric kidneys. Five patients had been transplanted before (nos 8, 12, 16, 17, 18). The graft had been removed in all these patients after cessation of graft function. Acute rejection episodes were diagnosed within the first 2 months after transplantation, one episode each in six patients (nos 2, 4, 11, 15, 16, 19) and two episodes in one patient (no 12). Signs of chronic rejection, i.e. steadily declining graft function or heavy proteinuria, were present in three patients (nos 7, 18, 19). Bilateral

nephrectomy was performed in all patients within the first 3 months after transplantation The original renal diseases were chronic pyelonephritis (nos 2 5 6 8 9 10 18) chronic glomerulonephritis (nos 3 7 12) polycystic disease of kidney (nos 11 13 15) hereditary nephritis (Alport) (nos 1 17) diabetic nephropathy (no 19) congenital renal hypoplasia (no 16) tuberculosis renis (no 4) and renal vascular occlusion (no 14)

The following groups of normotensive healthy control subjects were studied 1) PRC was measured in 12 subjects 10 males and 2 females mean age 26±5 years 2) PAC was measured in 12 subjects 6 males and 6 females mean age 49±20 years 3) renal hemodynamics were measured in 21 subjects 13 males and 8 females mean age 42±16 years

All patients and controls were told about the nature of the study before the tests and gave their informed consent to participation

Procedure

All patients were hospitalized for 12 days Blood samples for PRC and PAC were drawn in the morning at 8 a m in the supine position after the patients had been in bed all night and had fasted for approximately 8 hours PRC and PAC were measured on the day after admission after a 6-day period on a fixed low sodium intake (10 mEq/day) and after a further 6-day period when the sodium restricted diet was supplemented with sodium chloride tablets (150 mEq/day) On the day after admission PRC and PAC were also measured after one hour in the erect position

Renal clearances were performed at the end of each of the two dietary periods

BP was measured with sphygmomanometer after at least one hour in the supine position BP values used for the statistical calculations are mean values of at least three measurements

METHODS

PRC was measured using the method described by Giese et al (6) This involves radioimmunologic measurement of angiotensin I after previous dialysis of plasma incubation at 37°C and pH 7 4 with and without addition of an internal standard of human renin and extraction of the angiotensin I produced Medical Research Council Division of Biological Standards National Institute for Medical Research Mill Hill London placed Renin Human 68/356 at our disposal PRC is given in μ Goldblatt units (μGU)/ml plasma using the above mentioned standard human renin as reference J Giese M D Department of Clinical Physiology Amtssygehuset Glostrup Denmark provided antiserum against angiotensin I The coefficient of variation (day to-day) was 11%

PAC was measured using Damkjær Nielsen s method (25) This is a radioimmunologic measurement performed on plasma after previous extraction with dichloromethane purification on silica gel columns and chromatographic separation on paper The position of aldosterone on the paper strips was located by scanning

Aldosterone antiserum was obtained from Research Plus Laboratories Denville New Jersey The coefficient of variation (day to-day) was 15% PAC in samples from the same patients was determined within the same analytic run

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured using a constant infusion technique The reference substances employed were ^{131}I hippuran and ^{125}iothalamate (Amersham Radiochemical Pharmaceuticals) A priming dosage was given so that the plasma activity range was 300–600 cpm/ml for ^{131}I hippuran and 800–1600 cpm/ml for ^{125}iothalamate Plasma activity was kept stable by constant infusion with the aid of a Holter infusion pump Each patient was examined for 3 or 4 clearance periods of approximately 30 min duration Blood specimens were taken 5 min before the middle of each period Bladder catheterization was not performed Patients voided in either the standing or the sitting position During the course of 1 hour immediately prior to examination 1000 ml water was given to each patient and during the actual clearance periods a further 500 ml/hour The coefficient of variation between the clearance periods was less than 10% for all patients Earlier investigations (18 28) have shown that ^{131}I hippuran and ^{125}iothalamate are reliable substances for measurement of renal plasma flow and GFR

The Mann Whitney test Wilcoxon s signed rank test and Spearmann s test were used for the statistical calculations The values are given as mean ±1 S D

RESULTS

Plasma renin concentration

PRC in group 2 was 52±37 μGU/ml and was thus very similar to PRC in group 1 47±25 μGU/ml PRC in the non transplanted control group was 38±17 μGU/ml and did not differ significantly from either of the two transplanted groups ($p>$ 0 05) PRC was slightly to moderately elevated in one patient (no 6) in group 1 and in two patients (nos 10 12) in group 2

PRC did not change significantly in either group after one hour in the erect position (Fig 1) In group 1 PRC changed from 47±25 to 49±16 μGU/ml ($p>0$ 05) and in group 2 from 52±37 to 56±47 μGU/ml ($p>0$ 05) This is in contrast to the non transplanted control group in which PRC increased from 38±17 to 67±25 μGU/ml ($p<0$ 01) after one hour in the erect position

Table II shows the influence of the dietary procedures In group 1 PRC increased from 47±25 to 82±19 μGU/ml ($p<0$ 02) when the sodium intake was 10 mEq daily during the first period whereas a significant fall to 34±8 μGU/ml ($p<0$ 02) was measured after the second period on a 150 mEq

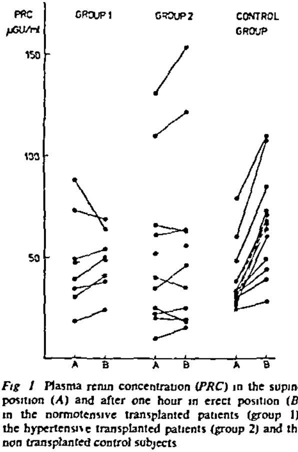


Fig 1 Plasma renin concentration (*PRC*) in the supine position (*A*) and after one hour in erect position (*B*) in the normotensive transplanted patients (group 1) the hypertensive transplanted patients (group 2) and the non transplanted control subjects

daily sodium intake In group 2 a significant increase in PRC from 52±37 to 69±32 µGU/ml ($p<0$ 01) was measured after the first period and a significant decrease to 34±19 µGU/ml ($p<0$ 01) after the second No significant differences were detected between the groups when PRC was compared after each of the two periods ($p>0$ 05)

Plasma aldosterone concentration

In group 1 PAC 7 8±2 8 ng/100 ml did not differ significantly from PAC in group 2 8 6±5 3 ng/ml ($p>0$ 05) These values were similar to those in the non transplanted control subjects 11 5±4 2 ng/ml ($p>0$ 05)

In group 2 a significant increase in PAC from 8 6±5.3 to 26 4±15 9 ng/100 ml ($p<0$ 01) was measured after one hour in the erect position whereas the corresponding values in group 1 were 7.8±2.8 and 15 1±7 2 ng/100 ml This difference was not significant ($p>0$ 05) but PAC increased in all but one patient (no 2)

Table II shows the influence of dietary sodium intake In group 1 PAC increased from 7.8±2.8 to 18 0±5 2 ng/100 ml ($p<0$ 05) after the first period and decreased to 5 2±1 9 ng/100 ml ($p<0$ 05) after the second In group 2 PAC increased from 8 6±5 3 to 16 6±11 7 ng/100 ml ($p<0$ 01) after the first period and decreased to 6 6±4.5 ng/100 ml ($p<0$ 01) after the second No significant differences were detected between groups 1 and 2 when PAC was compared after each of the two periods ($p>0$ 05)

Renal hemodynamics

Table III shows BP GFR RPF and filtration fraction (FF) in groups 1 and 2 at the end of each of the two dietary periods

RPF was 195±91 ml/min in group 2 which is significantly lower ($p<0$ 02) than the value of 356±154 ml/min measured in group 1 after the first period The corresponding values after the second period also differed significantly, 192±87 for group 2 and 347±137 ml/min for group 1 ($p<0$ 0?) When

Table II *PRC (µGU/ml) and PAC (ng/100 ml) before sodium restriction (A) after the period on 10 mEq sodium intake daily (B) and after the period on 150 mEq sodium intake daily (C)*

Pat no	PRC A	PRC B	PRC C	PAC A	PAC B	PAC C
Group 1						
1	49	79	33	7 7	11 6	4 4
2	18	94	18	9 0	19 3	5 8
3	73	81	44	6 0	13 5	5 2
4	39	63	39	–	–	–
5	34	56	38	7 0	25 4	2.0
6	88	109	34	12 6	16 3	5 8
7	30	94	30	4 7	21 9	7.8
Mean	47	82	34	7 8	18 0	5.2
S D	25	19	8	2 8	5 2	1 9
Group 2						
8	22	50	23	2 0	4 5	2.0
9	51	78	68	5 1	7 5	4 8
10	131	120	29	18 2	41 4	7 6
11	10	26	7	4 9	11 6	3 7
12	110	116	63	7 8	11 4	2.7
13	34	63	21	10 8	15 6	5.2
14	20	31	17	–	–	–
15	25	36	29	10 1	25.5	15.3
16	40	61	31	5 9	9 3	3 0
17	51	103	26	–	–	–
18	61	66	54	5 1	9 6	12.5
19	66	74	41	16 3	29 2	9.2
Mean	52	69	34	8 6	16 6	6 6
S D	37	32	19	5 3	11 7	4 5

Table III *BP (mmHg) GFR (ml/m n) RPF (ml/m n) and FF after the per od on 10 mEq sod um da ly (A) and after the per od on 150 mEq da ly (B)*

Pat. no	BP		GFR		RPF		FF	
	A	B	A	B	A	B	A	B
G oup 1								
1	110 65	110/75	58	74	379	325	0 176	0 228
2	131/90	119 85	49	49	196	174	0 250	0 282
3	104/73	101/70	107	102	589	502	0 182	0 03
4	105/70	110/70	128	128	541	556	0.237	0 230
5	116/78	111/78	70	70	311	291	0.225	0 241
6	123/68	121/70	88	94	322	339	0 273	0 277
7	176/95	185/96	43	46	201	244	0 214	0 189
Mean	124/77	122/78	78	80	356	347	0.222	0 236
S D	25/11	28/10	32	30	154	137	0 035	0 035
G oup 2								
8	1 4/100	127/100	37	35	160	137	0 231	0 256
9	163/105	135 95	67	67	244	247	0.275	0 271
10	170/110	150 100	70	75	226	204	0.310	0 368
11	162/111	155/113	65	61	251	227	0.259	0 269
12	183/100	190/105	76	76	369	343	0 206	0 222
13	149/110	152/114	68	68	298	315	0.228	0 216
14	190/108	185/108	66	69	194	218	0.340	0.317
15	190/130	211/130	54	59	146	163	0.370	0 362
16	155/100	181/111	40	45	163	168	0 245	0 268
17	165/110	160/110	34	31	14	131	0 239	0 237
18	189/100	210/110	8	10	18	23	0 444	0 435
19	178/100	190/105	36	38	124	127	0 290	0 299
Mean	168/107	171/108	52	53	195	192	0 286	0 293
S D	20/9	28 9	21	21	91	87	0 069	0 066

the three patients with chronic rejection were ex cluded RPF was still s gnif cantly lower ($p<0\ 05$) in the hypertensive group RPF in the control group 410±117 ml/m n d d not differ signif cantly from the value in group 1 ($p>0\ 05$) but was s gn f icantly higher than *in group* 2 ($p<0\ 01$)

After the first period GFR was 78+32 ml/min in group 1 and 52+21 ml/m n in group 2 ($p>0\ 05$) and after the second period 80+30 ml/min for group 1 and 53+21 ml/min for group 2 ($p<0\ 05$) In the control group GFR was s gnif cantly higher 108± 28 ml/m n than both in group 1 ($p<0\ 05$) and 2 ($p<0\ 01$)

FF was *significantly higher in group* 2 than *in* group 1 after the f rst period the values being 0 286+0 069 and 0 222+0 035 respectively ($p<0\ 05$) No s gnif cant d fferences however were measured after the second period 0 293+0 066 for group 2 and 0 236+0 035 for group 1 ($p>0\ 05$) W thin both groups no s gnif cant changes n renal hemodynam cs were induced by d fferent sod um intake ($p>0\ 05$)

The d etary procedures d d not cause any s gnif icant changes in BP the BP levels in group 1 being 124/77+25/11 and 122/78+28/10 mmHg ($p>0\ 05$) and in group 2 168/107+20/9 and 171/108+28/9 mmHg ($p>0\ 05$) after the first and after the second period respectively

Relationsh p between PRC PAC renal hemodynam cs and BP

PRC and PAC were not correlated with each other in any of the groups e ther before or after any of the d etary periods ($p>0\ 05$)

No s gnif cant correlation was found between BP and PRC or PAC in e ther of the groups either before or after any of the dietary periods ($p>0\ 05$)

Mean BP was s gnif cantly correlated to RPF in the normotensive group both after the first die tary period (rho −0 821 n 7 $p<0\ 05$) and after the second (rho 0 902 n 7 $p<0\ 02$) In the hypertensive group mean BP was not correlated to RPF e ther after the first period (rho 0 170 n 12 $p>0\ 05$) or after the second (rho 0 297 $n=12$ $p>0\ 05$)

PRC was correlated to RPF in the

Table IV *Urinary sodium excretion (mEq/day) before sodium restriction (A) after the period on 10 mEq sodium intake daily (B) and after the period on 150 mEq sodium intake daily (C)*

	A	B	C
Group 1			
1	86	36	139
2	155	12	141
3	106	2	130
4	155	17	197
5	51	16	189
6	79	22	140
7	105	16	189
Mean	105	17	161
S D	39	10	29
Group 2			
8	125	17	165
9	83	7	160
10	117	5	221
11	84	34	148
12	79	47	174
13	88	5	87
14	136	39	184
15	91	45	152
16	179	25	122
17	60	45	136
18	143	78	123
19	110	24	169
Mean	108	31	153
S D	34	22	34

group on a 150 mEq daily sodium intake (rho= 0 857 $n=7$ $p<0\ 05$), but not in the hypertensive group (rho=−0 082, $n=12$ $p>0\ 05$)

Relationship between dosages of prednisone and PAC or BP

o relationship was found between the dosages of nisone and PAC or BP in either of the two ps ($p>0\ 05$)

Urinary sodium excretion

Table IV shows urinary sodium excretion in the two groups In group 1 urinary sodium excretion de creased from 105±39 to 17±10 mEq/day after the first period ($p<0\ 02$) and increased to 161±29 mEq/day after the second ($p<0\ 02$) In group 2 the level 108±34 before sodium restriction decreased to 31±22 after the first period ($p<0\ 01$) and in creased to 153±34 mEq/day after the second ($p<0\ 01$) No significant differences were found be tween groups 1 and 2 when urinary sodium excre tion was compared after each of the two periods ($p>0\ 05$)

DISCUSSION

The role of the renin–aldosterone system in the development of posttransplant hypertension has not yet been fully clarified In our study PRC in the hypertensive group was very similar to the value found in both the normotensive group and the controls Only a slight elevation in PRC was found in one of the normotensive and two of the hyper tensive transplanted patients It has earlier been reported that PRC is increased in grafted patients during acute rejection episodes (11 17 24 30 37) During stable graft function several months after transplantation however normal values were found in most of our patients This is in good agreement with the results of others (2 21 26 27) Erect position did not induce any changes in PRC Other studies (9 21 26) have shown a significant increase in PRA in patients erect for four hours The lack of increase in our study group may be related to the fact that PRC was measured after only one hour in the erect position It is however remarkable that PRC increased considerably in the non transplanted control subjects after one hour in the erect position Thus the response to standing position appears to be abnormal in transplanted patients but according to our studies there are no differences between the hypertensive and the normotensive transplanted patients This abnormal response could possibly be attributed to the lack of renal innervation in transplanted patients The elevation of PRC after sodium restriction agrees with the results of others (1 9 16) Bennett et al (1) found higher PRA in a hypertensive group of transplanted patients than in a normotensive group with a daily intake of 10 mEq sodium whereas no difference was found when the sodium intake was 200 mEq daily This is in contrast to our results We found no changes in PRC between the groups after either of the two dietary periods The di vergence may be attributed to the presence of the recipients own kidneys in some of the patients of Bennett et al (1) and the fact that PRA was measured in blood samples from the graft vein Our results show that the renin content in venous plasma and the changes in PRC induced by dietary procedure are normal in most patients with post transplant hypertension whereas the response in PRC to erect position is suppressed in transplanted patients

The role of the mineralocorticoid hormones as a

cause of posttransplant hypertension is much disputed The study of Bennett et al (1) has shown elevated urinary aldosterone excretion in both a normotensive and a hypertensive group of patients on a diet containing 10 mEq sodium a day while the aldosterone excretion was suppressed to normal values when the diet contained 200 mEq sodium daily Sampson et al (26 27) also found elevated urinary aldosterone secretion rates in hypertensive grafted patients on an unrestricted sodium intake and suggest that the moderate hypertension seen after renal allotransplantation is related to an inappropriate production of aldosterone by the adrenal gland The plasma content of aldosterone has been measured by Cooke et al (4) who found normal PAC in 11 normotensive posttransplant patients This agrees with the results in the present study where normal values of PAC were found in both the normotensive and the hypertensive group The influence of dietary alterations on PAC in this study is similar to alterations of urinary excretion of aldosterone reported by Bennett et al (1) As in normals (33) a significant increase in PAC was measured after the patients had been in the erect position Thus our results show that PAC is normal in most patients with posttransplant hypertension and the changes in PAC during different sodium intakes and following changes in posture are very similar to the well known changes in control subjects

RPF was decreased in the hypertensive transplanted patients Our findings agree with the results of others (1 10) who found a decreased renal cortical blood flow with Xenon or Crypton wash out technique When the three patients with chronic rejection were excluded the RPF was still significantly lower in the hypertensive group BP elevation in transplanted patients is thus accompanied by changes in renal hemodynamics which are very similar to those in patients with essential hypertension (8 13 15) These alterations are usually attributed to increased vascular resistance in the glomerular arterioles It has not been clarified whether these changes are secondary to the elevated BP in essential hypertension or whether the initiating factors in hypertension are due to some alterations in the renal vascular bed which are subsequently aggravated by the increased BP In grafted patients however changes in renal hemodynamics may be related to vascular damage caused by minor subclinical rejections

As shown by others (26 27) no significant relationship was detected between PRC and PAC and we found no correlation between BP and PRC or PAC A remarkable divergence however was found between the normotensive and the hypertensive grup concerning the relationship between RPF and mean BP or PRC In the normotensive group RPF was significantly correlated to both mean BP and PRC, whereas no such relationship was found in the hypertensive group This suggests that although PRC is normal in most patients with stable graft function an abnormal regulation of renin secretion may be important for the sustained elevation of BP after renal allotransplantation

The study of Popovtzer et al (24) has shown a correlation between BP and the dosage of glucocorticoid hormones when graft function was stable Their study was performed within the first two months after transplantation and is not easily compared with ours since prednisone doses were considerably higher The theory about the dominant influence of glucocorticoid hormones for post transplant hypertension was supported by the results of Sampson et al (26) who found increased aldosterone secretion rates in patients studied ½–2½ years after transplantation and showed that injected ^{14}C labelled cortisone acetate could be converted to aldosterone Contrary to these results we found normal PAC and no relationship could be demonstrated between BP and either PAC or dosages of steroids We found no difference in steroid dosages between the groups Thus according to our results the importance of prednisone dosages for the development of posttransplant hypertension seems doubtful

Some authors (5 31) have described hypertension associated with hypercalcemia All patients in our study however had a normal serum calcium

ACKNOWLEDGEMENTS

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BOOK REVIEW

Drug-induced blood disorders By G C. de Gruchy 204 pp. £7.25 Blackwell Oxford 1975

Professor G C. de Gruchy died in Oct. 1974. In the years before his death he managed to complete his book on drug-induced blood disorders, apart from one chapter on sideroblastic anaemia. This unusually well written, easily read monograph is recommended for all prescribing physicians. It is a serious memento, since 17% of drug-induced blood disorders are the cause of fatal disease. The book gives an excellent survey of the diagnosis, clinical picture, treatment, pathogenesis, prophylaxis and laboratory findings [illegible] conditions as aplastic anaemia, agranulocytosis, [illegible] pure red cell anaemia, haemolytic [illegible] including the variety in patients with enzyme[illegible] es and megaloblastic anaemia.

It is a well known fact that chloramphenicol may cause aplastic anaemia, often with a fatal outcome, but it is probably less well known that chloramphenicol-induced aplastic anaemia may sometimes be followed by acute myeloblastic leukaemia. In the chapter on drug-induced agranulocytosis it is noted that the mortality rate is about 20% which is an alarming figure considering th[illegible] marrow usually returns to normal within 7–14 [illegible] the cessation of medication if the patient has [illegible] meantime succumbed to a complicating infec[illegible] fact—like several others—calls attention to th[illegible] ance of a careful haematological control in al[illegible] which a drug which is known to be capable [illegible] neutropenia is prescribed. It is also emphasize[illegible] phenothiazines are among the most frequent [illegible] agranulocytosis. Special attention is directed [illegible] promazine, and it is stated that thiouracil [illegible] is also one of the more frequent causes of agra[illegible] sis. Among the drugs which may give rise to sev[illegible] disorders, the author mentions other [illegible] methacin, antibiotics, sulphonamides, [illegible] butazone derivatives, antidiabetics, gold [illegible] and several others. On the other hand, anti[illegible] cytostatics are not discussed.

Professor de Gruchy's book is warmly recom[illegible] to all physicians and drug houses.

[illegible]

Acta Med Scand 200 5(9–5 2 (9?6

Thyroid Autonomy (Plummer s ase) with Contralateral Malignancy—Mere de[illegible]

Jan D Wiener and Ernst L Frensdorf

From the Department of Medicine Free University Hospital and the Depa[illegible] Onze Lieve Vrouw Gasthuis Amsterdam the Netherlands

[illegible]ACT A patient with an autonomously func[illegible] nodule in the left lobe and a papillary carci[illegible] n the right lobe of the thyroid gland is de[illegible] Some evidence suggests the association to be [illegible]an coincidental

[illegible]sociation of localized autonomous function [illegible]er s disease) and malignancy in the thyroid [illegible]ay be coincidental At all events it seems [illegible]ufficiently rare—at least in our region—to [illegible]he presentation of a new case We recently [illegible]patient in whom some findings suggest a [illegible]on from autonomy to malignancy

CASE REPORT

[illegible]ar-old woman was first referred to the Division of [illegible] Medicine of the Free University Hospital in June [illegible]cause of the presence of two thyroid nodules [illegible] 1×1 cm (right lobe) and 2×1 cm (left lobe) [illegible]vely Apart from increased perspiration signs and [illegible]s of hyperthyroidism were absent A history of [illegible] therapy could not be elicited

[illegible] thyroxine radioiodine uptake and absolute up[illegible] re normal but serum triiodothyronine (T_3) was [illegible]/l (normal 1 55–3 1 nmol/l) indicating T_3 hyper[illegible]ism Scintiphotography with ^{99m}Tc pertechnetate [illegible]nd after suppression with T_3 showed the nodule [illegible]eft lobe to be autonomously hyperfunctioning [illegible] the other nodule could not be delineated on the [illegible]ig 1)

[illegible] nodules were excised surgically The scinti[illegible]lly inconspicuous nodule of the right lobe [illegible] 2 5 g Within a fibrous pseudocapsule it was [illegible]composed of papillary structures which must be [illegible]ed as papillary carcinoma There was fluent [illegible]n from normal follicular epithelium (Fig 2) The [illegible]ously functioning lesion of the left lobe was a partially encapsulated nodu[illegible] [illegible] signs of malignancy were seen [illegible] an area with atypical larger [illegible] chromasia (Fig 3)

Total thyroidectomy was then [illegible] further signs of malignancy were [illegible] later whole body scanning (3 days after [illegible] onstrated the presence of four very small [illegible] the neck presumably rests of the gland itself [illegible] evidence of functioning metastases was found

COMMENT

Thyroid malignancy and functional autonomy can occur simultaneously in several ways

Malignant (metastasizing) autonomous nodules are extremely rare in man (1 10) in contrast to the dog (16) although autonomously functioning (and growing) lesions might be considered as—benign—neoplasms (7)

A carcinoma in near or contralateral to a hyper active nodule has been found in a greater number of cases Thus (17) comparing pathologic findings in nodules with different functions listed data from 31 published series omitting one of these (insuffi cient data) and adding six series of autonomous lesions (Plummer s disease) (2 3 7 9 14 20) yields a total of 29 cancers in about 1200 cases (2 4%) This figure should be considered as only a rough approximation of the true incidence for a number of reasons Firstly some scintigraphically hyper active nodules are not autonomous but only thicker than the paranodular tissue (12 18) Whether the incidence of malignancy in these cases is similar to that in Plummer s disease remains to be established Secondly some series contain only patients with

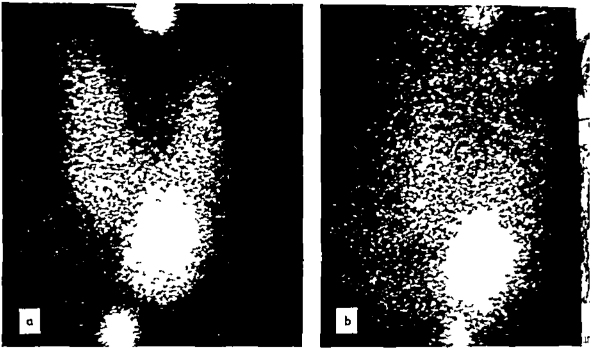


Fig 1 Scintiphoto (*a*) before and (*b*) during suppression with T_3 The nodule in the left lobe is shown to be autonomously hyperfunctioning the one in the righ[t] is not visualized on either scan

solitary nodules whereas in others multinodular goitres are included Although these may be two variants of the same disorder Plummer s disease without fundamental pathogenetic difference (4 13 19) the incidence of malignancy is not necessarily equal in these glands Thirdly patient selection may play a role surgically treated patients may differ in this respect from those treated with ^{131}I or left untreated Fourthly, a number of malig[nant] lesions has probably remained undetected bec[ause] in many cases only the hyperactive nodule was [ex]cised Prior irradiation may on the other h[and] have induced some of the cancers and pos[sibly] some of the autonomous lesions as well (7)

Two other aspects should be mentioned In r[...] of the cases where sufficient data are available[...]

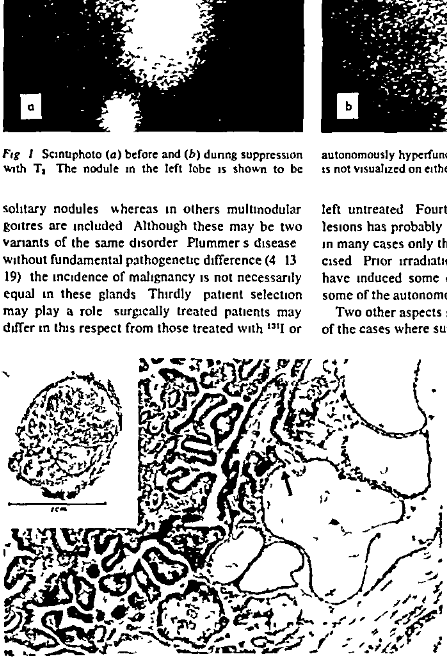


Fig 2 Nodule of the righ[t] lobe well encapsulated b[y] fibrous pseudocapsule T[he] transition of follicular ep[i]thelium to atypic papillar[y] structures is indicated by arrow



F \ A
I b
ed A
c

les ons were small occult pap llary These also occur in a non negl g ble on of cl n cally normal thyro d glands n one post mortem series (15)) Separately e mal gnant and autonomous nodules in one h as found n the present pat ent appear e besides several of these pat ents h story of previous irrad ation po nt is the poss b l ty that fferences both in the inci and n the assoc at on fourth to one th rd d sm have Plum 9) and th s compares well with gs in o tne n Germany and Switzerland (9) dney Austral n the other hand the in ce h been reported as less than 3% (6) Pub Am can f gures l e some here in between nted out by Hamburger (7) the nc dence of nanc n Plummer s d sease exceeds 10% in a er of American series but appears to be con ably less n Europe In f ve large European totall ng about 500 operated patients (2 3 9 l) t as only 1 2%

there more than a chance relat onsh p between mer s d sease and thyro d mal gnancy? The r can data c ted above suggest that th s may be ase In Europe on the other hand the sporad c at on of both les ons s compat ble with mere dence In our case however the h stolog c ngs support a h stogenet c relationsh p between wo nodules In both there was fluent transi tion from morpho n t morphic follicular ep the format on of pap llary carc nom i process the ep thel um may lose uncti r ity by gain ng proliferative tendency The nomously funct on ng nodule could mark a stage n such a progress w th pers st ng funct onal acti y while hormonal feedback control had been lost

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BOOK REVIEW

Drug-induced blood disorders By G C de Gruchy 204 pp £7 25 Blackwell Oxford 1975

Professor G C de Gruchy died in Oct 1974 In the years before his death he managed to complete his book on drug-induced blood disorders apart from one chapter on sideroblastic anaemia This unusually well written easily read monograph is intended for all prescribing physicians It is a serious memento since 17% of drug-induced blood disorders are the cause of fatal disease The book gives an excellent survey of the diagnosis clinical picture treatment pathogenesis prophylaxis and laboratory findings in such conditions as aplastic anaemia agranulocytosis thrombocytopenia pure red cell anaemia haemolytic anaemia including the variety in patients with enzymopathies and megaloblastic anaemia

It is a well known fact that chloramphenicol may cause aplastic anaemia often with a fatal outcome but it is probably less well known that chloramphenicol-induced aplastic anaemia may sometimes be followed by acute myeloblastic leukaemia In the chapter on drug induced agranulocytosis it is noted that the mortality rate is about 20% which is an alarming figure considering that the marrow usually returns to normal within 7-14 days the cessation of medication if the patient has not meantime succumbed to a complicating infection fact—like several others—calls attention to the ance of a careful haematological control in all c which a drug which is known to be capable of i neutropenia is prescribed It is also emphasized t phenothiazines are among the most frequent ca agranulocytosis Special attention is directed to promazine and it is stated that thioridazine (M is also one of the more frequent causes of agranu sis Among the drugs which may give rise to sever disorders the author mentions phenylbutazone methacin antibiotics sulphonamides tuberculo hydantoin derivatives antidiabetics gold barb and several others On the other hand antine cytostatics are not discussed

Professor de Gruchy s book is warmly recom to *all* physicians (and drug houses)

J Bichel Højbjerg D